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DINYA MARIANN KATALIN

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Programvezető: Dr. Antal István, egyetemi tanár

Konzulens: Dr. Mórotz Gábor, tudományos munkatárs

THE ROLE OF THE DISEASE-SPECIFIC MICROENVIRONMENT IN THE DESIGN OF BIODEGRADABLE POLYMERIC DRUG DELIVERY SYSTEMS

PhD thesis

Mariann Dinya

Semmelweis University Doctoral College

Pharmaceutical Sciences and Health Technologies Division

Supervisor: Gábor M. Mórotz, PhD

Official reviewers: Miléna Lengyel, PharmD, PhD
László Molnár, MD, PhD

Head of the Complex Examination Committee: Kornélia Tekes, DSc

Members of the Complex Examination Committee: Éva Sátory, DSc
Mihály Kata, DSc

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List of Abbreviations

ABAH: 4-aminobenzoic acid hydrazide

MOD: mean optical density

MOF: metal-organic framework

PCT: porphyria cutanea tarda

PEG: poly(ethylene glycol)

PEG-PPS: poly(ethylene glycol)-block-poly(propylene sulfide)

PLA: poly(lactic acid)

PLGA: poly(lactic-co-glycolic acid)

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RBC: red blood cell

ROS: reactive oxygen species

SD: standard deviation

SEM: standard error of the mean

SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation

1. Introduction

The effectiveness of targeted and controlled drug delivery is determined to a decisive extent by how well the carrier system can adapt to the local biochemical and physicochemical environment in which release and therapeutic action must occur. In contemporary drug carrier development, the emphasis has therefore shifted away from the mere generation of new carriers and increasingly toward understanding their microenvironment-specific behavior. In this context, disease-associated microenvironmental alterations are not secondary circumstances, but one of the defining organizing principles of formulation design, particularly in the more personalized therapeutic strategies of the future. For a long time, research in targeted drug delivery focused primarily on describing new carrier systems, stimulus-responsive mechanisms, and individual formulation solutions, while much less attention was paid to whether recurrent patterns could be identified behind successful systems that directly link polymer selection to the pathological microenvironment of the disease. As a result, polymer selection in practice is often empirical, although growing evidence suggests that the behavior of carrier systems is fundamentally determined by the local environment in which they must be activated, remain stable, and subsequently release the active agent. [1-7]

In this context, the pathological microenvironment is not merely a passive background of disease but one of the primary organizing principles of the formulation landscape. pH, redox status, enzymatic activity, microbial enzymatic effects, ionic conditions, and, in certain indications, even mechanical stress or local flow characteristics are factors that, depending on polymer structure, may decisively influence swelling, degradation, changes in particle size, retention, targeting, and ultimately the kinetics of drug release. Consequently, the same polymer function may confer therapeutic benefit in one disease, while becoming irrelevant or even distinctly unfavorable in another pathological environment. The real question, therefore, is not whether pH-, ROS-, or enzyme-sensitive carrier systems exist, but which material-structural and functional solutions are favored by the dominant local driving forces of a given disease. [8-12] Recent preclinical literature has made it increasingly clear that the distribution of successful systems is not random. In inflammatory bowel diseases, methacrylate- and polysaccharide-based

systems repeatedly tuned to pH, ionic, and microbial stimuli emerge; in joint and cartilage disorders, enzyme-sensitive matrices, predominantly based on hyaluronic acid or collagen, predominate; in atherosclerosis and other oxidative inflammatory models, ROS- and shear stress-responsive platforms recur; while in tumor settings, composite systems responding to multiple stimuli and adapted to a heterogeneous microenvironment come to the fore. Thus, a structural relationship emerges among the polymer backbone, the activation mechanism, and the disease-specific microenvironment that extends beyond the level of individual formulation examples. This recognition shifts polymer selection away from technological routine toward decision-making organized on a pathophysiological basis. [13-20] The importance of the relationship between the microenvironment and polymer performance is also supported by earlier observations of our own. The behavior of the experimentally investigated matrix systems changed substantially even in response to relatively small variations in pH and ionic composition, indicating that polymer response is not solely a function of material composition itself but must be interpreted together with the physicochemical parameters of the surrounding medium. In parallel, human samples also showed that a pathological state may be associated with measurable alterations in the internal chemical environment. In porphyria cutanea tarda, the concentrations of several macro- and microelements in whole blood, as well as the pattern of correlations between these elements, differed from those of the control group, suggesting that the disease is associated not only with organ-level or molecular abnormalities but also with a broader microenvironmental reorganization. Also relevant was the animal experimental observation reflecting the role of the oxidative vascular environment, in which modification of myeloperoxidase activity was associated with structural alteration of the aortic wall. Taken separately, these results did not yield a general formulation model; taken together, however, they pointed in the direction that the characteristics of the pathological environment and the behavior of carrier systems are related in a non-random and interpretable manner. [21, 22] The present work undertakes a systems-level interpretation of this set of relationships. Its starting point is that our earlier experimental, human, and animal observations, from different perspectives, pointed to the same problem: the behavior of a carrier system cannot be separated from the pathological environment in which it must function. This recognition is extended through a systematic analysis of contemporary preclinical literature by examining, within

a single interpretative framework, the polymer classes, trigger mechanisms, and formulation logics that repeatedly associate with different disease groups. The central hypothesis is that the performance of biodegradable polymers is not a universal property but depends on their fit to the dominant pathological microenvironmental triggers. Consequently, mapping the recurrent polymer-trigger associations observed across different indications is not merely a descriptive exercise but a means of grounding a rational approach to disease-calibrated, microenvironment-based polymer selection. [23]

2. Objectives

The fundamental objective of the dissertation was to examine whether the selection and interpretation of biodegradable polymer carrier systems can be placed on a new basis if the central organizing principle of formulation is defined not by general technological considerations but by the characteristics of the pathological microenvironment of the target disease. The starting assumption was that the therapeutic performance of polymer-based carrier systems cannot be regarded as a general, indication-independent property, because it depends decisively on the extent to which the structural and functional characteristics of the carrier fit the local pathobiochemical environment in which drug release, retention, and therapeutic effect must be achieved.

Accordingly, the aim of the dissertation was to explore whether recurrent, system-level interpretable relationships can be identified among disease-specific microenvironmental conditions, polymer functions, and therapeutically effective carrier systems, relationships that shift polymer selection toward pathophysiological rationality. The central ambition of the work was to establish an interpretative framework in which the pathological microenvironment appears not as a passive background of disease but as a primary organizing principle of polymer selection.

The specific aim of the work was to determine how the dominant microenvironmental driving forces associated with different disease types can be linked to recurrently successful polymer classes, trigger mechanisms, and carrier-system types, and whether these relationships allow the formulation of generalizable conclusions that go beyond the level of individual reports.

The specific objectives of the dissertation were as follows:

1. Reinterpretation of the earlier in-house antecedents of the microenvironment-polymer relationship.

The first objective was to demonstrate that the relationship between the microenvironment and polymer function had already been recognized at an early stage on the basis of our previous experimental observations. The aim was not to provide a mere summary of earlier results, but to show that the pH- and ion-sensitive behavior of polymer

matrices, as well as their environment-dependent responsiveness, can be interpreted as direct antecedents of the central concept of the present dissertation.

2. Demonstration of the biological reality of the pathological microenvironment through human and animal experimental examples.

The second objective was to substantiate that the significance of the microenvironment is not confined to model systems or theoretical formulation considerations, but can also be captured at the human and animal experimental level. This required demonstrating that disease states may be accompanied by measurable rearrangements of the internal chemical environment, and that oxidative and inflammatory conditions may be associated with structural consequences at the tissue level.

3. Pattern-seeking processing of preclinical in vivo data.

The third objective was to process the available preclinical in vivo data on biodegradable, stimulus-responsive polymer carrier systems in a way that does not stop at describing individual reports but seeks to identify the structural relationships among them. The aim was to determine which polymer classes and which stimulus-responsive mechanisms recurrently associate with therapeutically effective systems across different disease models.

4. Identification of polymer-trigger patterns associated with the major disease groups.

The fourth objective was to explore which local microenvironmental factors favor specific polymer functions and carrier-system types in the major disease groups, particularly inflammatory bowel diseases, joint and cartilage disorders, cardiovascular inflammatory-oxidative conditions, and solid tumors. The aim was to delineate recurrent associations showing that certain polymer families and response mechanisms appear not randomly but consistently in specific indications.

5. System-level interpretation of recurrent associations among polymer composition, response mechanism, and disease category.

The fifth objective was to examine whether generalizable associations extending beyond the level of individual reports can be identified among polymer composition, stimulus-

responsive behavior, and disease category. The aim was not to establish a direct ranking of efficacy, but to demonstrate that the occurrence of polymer functions fitted to the pathological microenvironment displays a structured pattern, and that this structure reflects a pathophysiologically interpretable organizing principle.

6. Establishment of the concept of disease-calibrated, microenvironment-based polymer selection.

The final objective of the dissertation was to formulate a new interpretative framework in which polymer selection is not a technological routine but a rational decision adapted to the dominant microenvironmental characteristics of the disease. At the center of this view is the premise that the fit between the carrier system and the target disease is determined by local pathobiochemical conditions, dominant trigger mechanisms, and the structure of the therapeutic environment. The actual novelty of the dissertation can be condensed into this final objective: the disease microenvironment emerges as a system-level, disease-calibrated organizing principle of polymer selection.

3. Methods

The methodological structure of the dissertation is dual. On the one hand, it builds on a brief, focused summary of our previous experimental, human, and animal observations; on the other, it relies on the structured processing of contemporary preclinical literature. The purpose of this chapter is therefore not to re-present each earlier investigation in full detail, but to summarize those methodological elements that are required for interpreting the microenvironment-polymer relationship and the selection of polymers fitted to pathological environments. The structured literature review was reported in accordance with PRISMA 2020 [24], and the animal-study risk-of-bias framework was informed by SYRCLE [25]. Quantitative heterogeneity and alternative synthesis approaches were considered where applicable [26-28], while the detailed analytical workflow and dataset are reported in our polymer study [23].

3.1. Summary methodological framework of in-house experimental investigations

The early investigation of the relationship between the microenvironment and polymer function focused on matrices prepared from medium-viscosity Eudragit L 100-55 (Sigma-Aldrich) and sodium alginate. For the experiments illustrated in Figures 1, 2, 5a, 5b, and 7, the polymers were mixed in a 2:1 mass ratio. The matrices were examined in hydrochloric medium at pH 1.2 and in phosphate buffer media at pH 4.50 ± 0.05 and pH 6.80 ± 0.05. The dissolution procedure followed the rotating-basket method of the Pharmacopoeia Hungarica VII, using a Pharmatest PTW-II apparatus at 37 ± 0.5 °C and 100 rpm. The pH 4.5 medium contained 68.05 g KH_2PO_4 in 3750 mL distilled water; the pH 6.8 medium contained 34.0 g KH_2PO_4 and 4.6 g NaOH in 3750 mL distilled water, with final adjustment by 1 M NaOH. Matrix volume was calculated as $V = \pi r^2 h$. Swelling/volume ratio was expressed as V_t/V_0, where V_0 is the initial dry-matrix volume and V_t is the volume measured at time t; thus, a value of 1 denotes the initial volume. The reported values represent mean ± SD or mean ± SEM, as indicated; $p < 0.05$ was considered statistically significant. Calculations were performed using Microsoft Excel 2003 and Microcal Origin 6.0 [29, 30].

Within the human investigation line, whole-blood analysis was performed in samples from patients with porphyria cutanea tarda and from controls in order to characterize the chemical features of the pathological internal environment. In addition to routine laboratory parameters, 19 macro- and microelements were determined by inductively coupled plasma optical emission spectrometry. Samples were prepared by acid digestion, and the elemental analysis was complemented by group-level comparisons and correlation analysis. In the present work, the methodology of this investigation is used to support the notion that disease states may be accompanied by measurable alterations of the internal chemical milieu. [21]

Within the animal experimental line, the role of the vascular oxidative environment was investigated in a cholesterol-fed rabbit model. In this model, myeloperoxidase inhibitors were applied, and the morphological and histochemical characteristics of the aorta were analyzed. The examinations included determination of intimal plaque area, measurement of media thickness, estimation of smooth muscle cell density, and evaluation of peroxidase activity. In the dissertation, this methodological framework serves to demonstrate, through our own earlier findings, the functional significance of the oxidative and inflammatory microenvironment. [22]

3.2. Structured processing of contemporary preclinical literature

The central, system-level analytical part of the dissertation is based on the structured processing of contemporary preclinical literature on biodegradable, stimulus-responsive polymer carrier systems [23]. The literature search was conducted in the PubMed and Scopus databases using a predefined search strategy and was reported in accordance with PRISMA 2020 [24]. Search terms were organized around polymer-based drug carriers, stimulus-responsive mechanisms, and relevant inflammatory or tumor animal models. The study covered original articles published between 1 January 2020 and 31 August 2025. During study selection, only in vivo preclinical investigations were analyzed in which a biodegradable polymer carrier system was used and drug release or carrier activation was linked to a pathological microenvironmental trigger.

Excluded from the analysis were review articles, studies reporting exclusively in vitro results, works presenting non-biodegradable or non-polymeric systems, and developments in indications that did not match the research question of the present dissertation. The final analysis included 65 articles. Structured data extraction was performed from these studies, covering the disease model, polymer family, carrier type, primary stimulus mechanism, route of administration, and the nature of the reported therapeutic outcomes. The aim was not to establish a direct ranking of efficacy across different models, but to determine which polymer-trigger associations recur across different disease types. [23]

3.3. Classification of stimulus-responsive systems

The investigated systems were categorized according to the dominant activation mechanism reported in the respective articles. Accordingly, pH-responsive, ROS-/redox-responsive, enzyme-sensitive, ion-sensitive, and dual- or multi-stimulus-responsive systems were placed into separate groups. Classification was based not on the general chemical properties of the polymers but on the principal trigger mechanism emphasized in each article as relevant to in vivo function. This made it possible to compare carrier systems across different indications not only by material class but also by functional response mode. [23]

Disease categories were also established with reference to the dominant pathological environment. The major analytical groups comprised inflammatory bowel diseases, joint and cartilage disorders, cardiovascular inflammatory-oxidative conditions, and solid tumors. This categorization made it possible to examine which polymer solutions are associated with the same trigger mechanism in different indications and in which pathological environments certain polymer families appear consistently. [1-7, 13, 15, 18]

3.4. Processing and interpretative approach

Because of the heterogeneity of preclinical models, the primary approach was descriptive, systematic, and relationship-seeking [23]. Associations among polymer families,

polymer-chemical categories, dominant trigger mechanisms, and disease groups were examined by cross-tabulation, chi-square testing, Cramér's V, and standardized residual analysis. Direction-of-effect synthesis and vote-counting were used when pooled effect estimates were not appropriate [27]. Statistical heterogeneity was considered according to established principles [26], and potential small-study effects were considered where the available data permitted [28]. Because many studies did not report complete, directly comparable quantitative data, the focus was not on calculating pooled effect sizes, but on identifying recurrent disease-specific formulation patterns.

4. Results

4.1. Experimental and conceptual antecedents of the relationship between the microenvironment and polymer function

The experimental findings made it clear that the behavior of polymer matrix systems cannot be interpreted solely on the basis of material composition. Systems with identical or nearly identical compositions exhibited different swelling, volume-change, and release behavior in media of different pH and ionic composition, indicating that the functional performance of the carrier depends to a substantial extent on the characteristics of the surrounding medium. From the perspective of the present dissertation, the most important lesson of these observations is that polymer response cannot be regarded as a stable, environment-independent material property, but rather as the outcome of interactions with the medium.

The importance of pH- and ion-dependent behavior lies particularly in the fact that it already made visible, even in relatively simple model systems, that carrier function cannot be separated from the environment in which activation must occur. This recognition later became directly transferable to pathological microenvironments. If the composition of the medium is already decisive under model conditions, it may reasonably be assumed that local biochemical alterations associated with different diseases also selectively influence the performance of carrier systems in vivo. One of the starting points of the present dissertation is precisely this idea: the relationship between the polymer and the medium must be regarded not as a technological background issue, but as a fundamental component of formulation rationality.

The significance of polymer response to environmental stimuli can be captured in the fact that changes in pH, ionic composition, enzymatic effects, and other physicochemical factors may substantially modify swelling, structural rearrangement, and, through these changes, the characteristics of controlled drug release. [29, 30] This insight is particularly important for the present dissertation because it indicates that the behavior of carrier systems cannot be reduced solely to the intrinsic composition of the carrier material, but can only be interpreted together with its interactions with the surrounding medium.

This was also supported by earlier in-house experimental observations showing that relatively minor modifications in polymer composition may induce substantial changes in matrix response and drug-release kinetics even when the set of components remains unchanged and only their proportions are altered. All this suggests that the relationship between polymer behavior and environmental parameters cannot be described as a simplified binary interaction, but rather as a sensitive and finely tunable system of interactions whose true significance becomes particularly evident in the pathological microenvironment. Within the interpretative framework of the present work, this is of special importance because it illustrates that evaluating the performance of carrier systems requires not only a material description of the polymer, but also consideration of the local chemical and physicochemical conditions under which the given system exerts its effect.

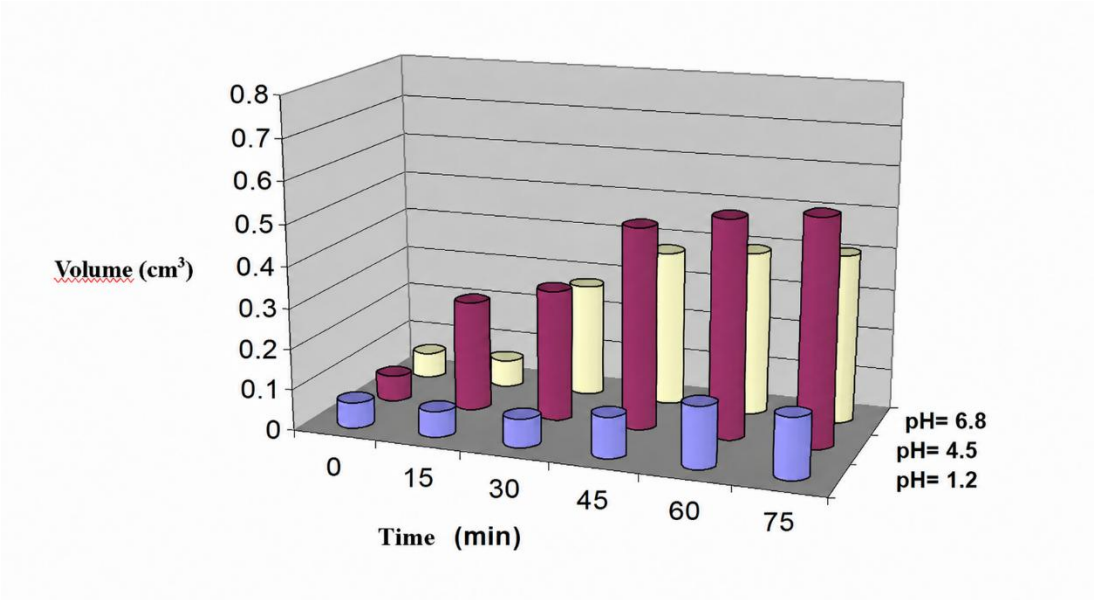

Figure 1. *Absolute matrix volumes measured in media of different pH for the Eudragit L 100-55-sodium alginate mixture (2:1, w/w). The media were hydrochloric medium at pH 1.2 and phosphate buffers at pH 4.5 and 6.8, prepared as described in Section 3.1. Source: author's own unpublished experimental data.*

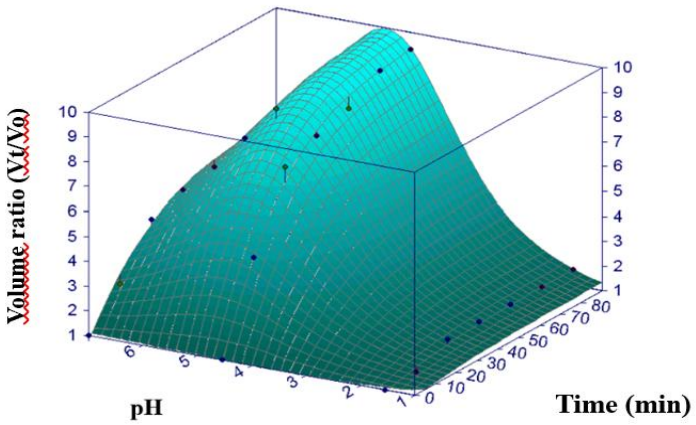

Figure 2. *Swelling/volume ratio (V_t/V_0) of the Eudragit L 100-55-sodium alginate system (2:1, w/w) in media of different pH. V_0 is the initial dry-matrix volume and V_t is the volume at time t. The greater apparent volume at pH 4.5 than near pH 6.8 is attributable to the combined pH- and ionic-composition-dependent hydration and structural rearrangement of the mixed polyelectrolyte matrix, rather than to pH alone. Source: author's own unpublished experimental data.*

Taken together, the two figures illustrate that, even at identical polymer composition, the pH and ionic composition of the medium can substantially modify system volume and swelling behavior; in other words, environment-dependent function is experimentally well captured.

4.2. Human observations on alterations of the pathological internal environment

One important lesson of the human results was that pathological conditions may be associated with measurable alterations in the internal chemical environment. In the elemental analysis of whole blood in porphyria cutanea tarda, the concentrations of several macro- and microelements differed from those of the control group. The most important differences were a significant decrease in magnesium, phosphorus, and sulfur levels and an increase in barium level, but no less important was the rearrangement of the correlation relationships among the elements. This suggested that the disease is associated not merely with isolated laboratory abnormalities, but with an organized modification of the internal chemical milieu. [21]

Within the interpretative framework of the dissertation, the significance of these human data lies not in rewriting the detailed pathomechanism of the disease in question, but in supporting the view that pathological states may be associated with measurable alterations of the internal chemical environment that can be integrated into the broader concept of the microenvironment. The modification of whole-blood elemental composition and correlation patterns indicates that the chemical environment associated with disease cannot be regarded as a static background. In this way, the human line of evidence creates a conceptual bridge between model systems and the disease-specific carrier systems discussed later.

	Control ($n = 6$) (µg/g)	PCT ($n = 8$) (µg/g)	Detection limit (µg/g)
Al	0.658 ± 0.313	2.748 ± 1.169	0.1
B	0.657 ± 0.493	2.402 ± 1.04	0.02
Ba	0.013 ± 0.005	0.108 ± 0.048*	0.002
Ca	53.64 ± 11.89	66.41 ± 26.79	0.002
Cu	0.738 ± 0.159	0.706 ± 0.130	0.01
Fe	530.4 ± 51.7	483.82 ± 51.18	0.01
K	1790 ± 183	1542.74 ± 225.06	0.5
Mg	41.37 ± 6.15	32.52 ± 5.96*	0.0025
Mn	0.105 ± 0.071	0.039 ± 0.015	0.005
Na	2323.00 ± 160.00	2270.01 ± 139.09	0.05
P	329.60 ± 18.31	297.22 ± 24.48*	0.25
S	1501 ± 83	1319.70 ± 145.49*	0.25
Zn	7.85 ± 0.99	6.79 ± 1.78	0.001

*Significant difference vs. controls.

Table 1. *Elemental determination values in healthy subjects and in the group with porphyria cutanea tarda. [21]*

By summarizing the principal elemental differences, the table illustrates that pathological conditions may be accompanied by an organized chemical rearrangement affecting multiple parameters.

4.3. Animal experimental observations in an oxidative vascular microenvironment

The animal experimental results made the functional significance of the vascular oxidative microenvironment visible. In the cholesterol-fed rabbit model, structural changes in the aortic wall and modification of peroxidase activity were observed following treatment with myeloperoxidase inhibitors. The increase in medial thickness in both plaque-free and plaque-containing areas suggested that modification of the oxidative and inflammatory environment may influence tissue structure as well. Together with the decrease in peroxidase activity, this indicated an interpretable relationship between redox status and the structural response of the vessel wall. [22]

Within the interpretative framework of the dissertation, these observations are significant because the oxidative microenvironment appears here not merely as a general pathological background, but as part of a biological process associated with structural consequences. This directly bridges to the logic of modern ROS-responsive cardiovascular platforms. If redox status is an organizing element of pathology, then the development of redox-sensitive carrier systems may be interpreted not as a technological idea, but as a solution fitted to the dominant local trigger. [8, 9, 15-17, 31-34]

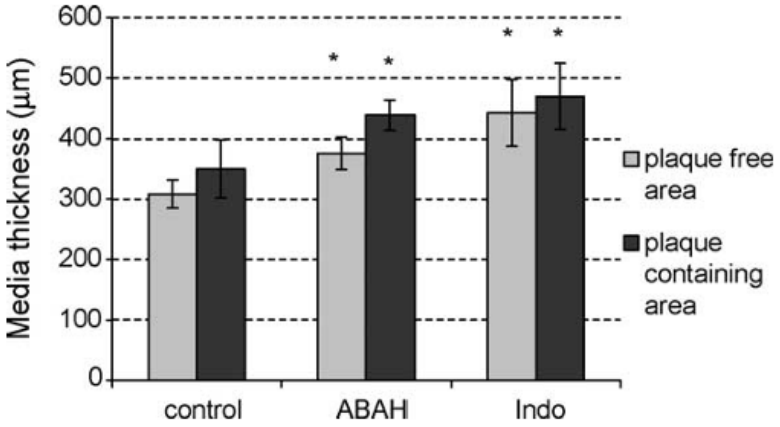

Fig. 3. Aortic media thickness of control (cholesterol-fed only) and ABAH or indometacin treated animals in plaque-free or plaque-containing areas. Data are means ± SEM. $*p < 0.05$.

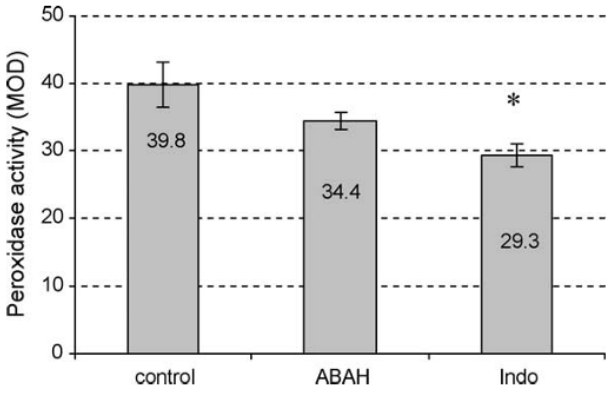

Figure 4. *Peroxidase activity in the aortic wall of control animals (fed a cholesterol-rich diet alone) and in ABAH- and indomethacin-treated animals. The bars indicate the mean optical density (MOD ± SEM) of the aortic media, reflecting the accumulation of peroxidase reaction products. $p < 0.05$. [22]*

Taken together, these two figures illustrate that modification of redox status may have detectable structural and enzymatic consequences in the vascular wall.

Figures 5a-8 present a qualitative morphological comparison between the environment-dependent rearrangement of the polymer matrix and the spatial distribution of atherosclerotic deposits:

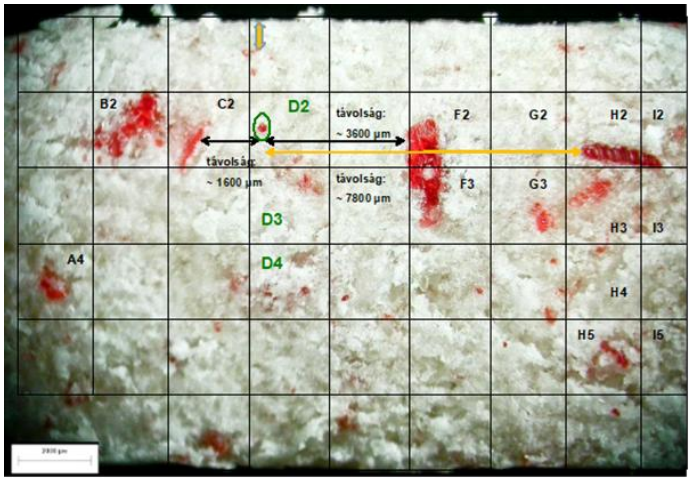

Figure 5a. *Polymer matrix tablet (Eudragit L 100-55-sodium alginate, 2:1, w/w) in the dry state before immersion in distilled water. The alphanumeric labels identify positions in the superimposed measurement grid. Solid arrows indicate measured distances between selected reference points. The red regions are Sudan Red-stained lipophilic marker particles distributed within the matrix. Source: author's own unpublished experimental image.*

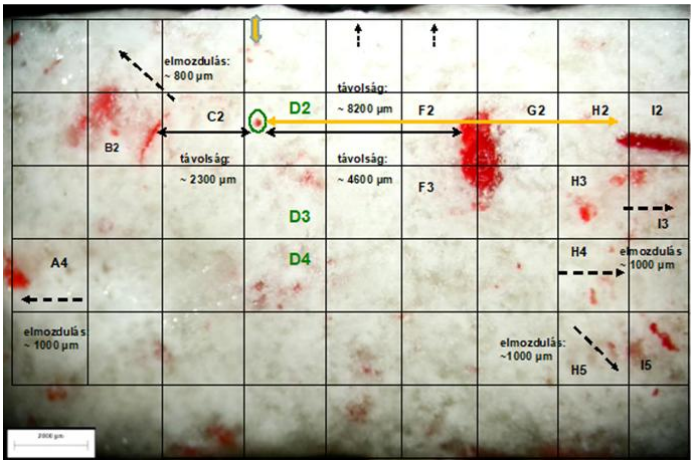

Figure 5b. *The same polymer matrix tablet after 15 minutes of swelling in distilled water. The alphanumeric labels identify the corresponding positions in the measurement grid. Solid arrows indicate measured distances, whereas dashed arrows indicate the direction and approximate extent of marker displacement. The red regions are Sudan Red-stained lipophilic marker particles. Their displacement results from water penetration, polymer hydration and swelling, and the consequent reorganization of the matrix. Source: author's own unpublished experimental image.*

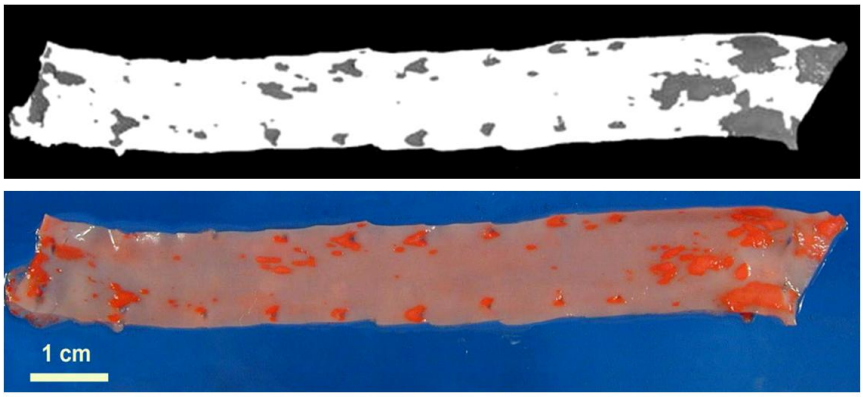

Figure 6. *Atherosclerotic aorta. Sudan Red staining clearly visualizes the plaques. [22]*

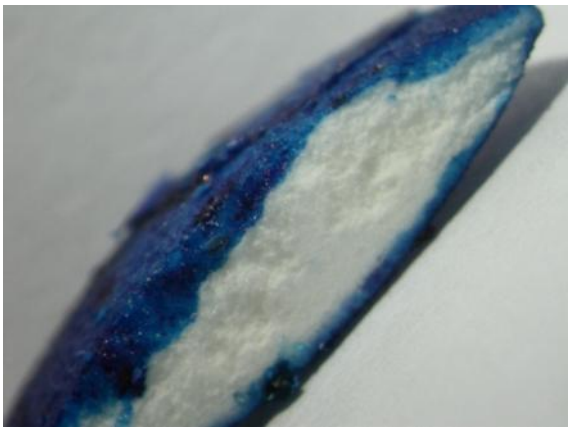

Figure 7. *Swelling front of the polymer matrix tablet (stained with methylene blue). Source: author's own unpublished experimental image.*

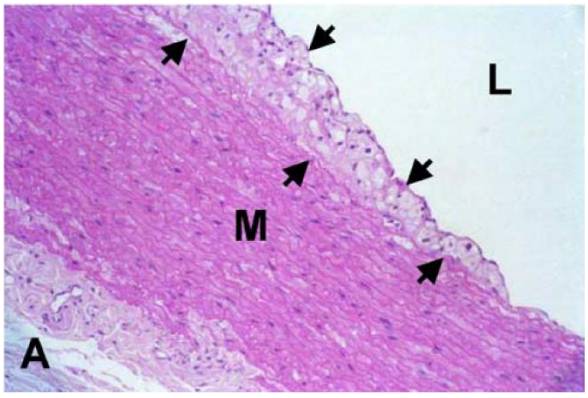

Figure 8. *Aorta of a rabbit fed a cholesterol-rich diet, with intimal deposits. L = lumen; M = media; A = adventitia. Arrows indicate atherosclerotic plaques/intimal deposits. [22]*

The figure series provides a qualitative, hypothesis-generating morphological comparison rather than a quantitative equivalence between the two systems. Figures 5a and 5b show that matrix rearrangement after contact with water is zonal: movement of Sudan Red-stained lipophilic marker particles and structural change are greatest in the hydrated peripheral region, while the center remains relatively stable. Figure 7 likewise shows a distinct swelling front in the polymer matrix. By comparison, Figures 6 and 8 show the spatially heterogeneous distribution of atherosclerotic deposits in the vessel wall. No statistical image-matching analysis was performed, and the images are not presented as proof of a shared mechanism. Their value is conceptual: both demonstrate that surface contact and a local microenvironment can generate spatially organized rather than uniform structural responses. This observation may guide future formulation studies, in which the proposed parallel should be tested by quantitative image analysis, predefined spatial descriptors, and correlation with release or retention parameters.

4.4. Disease-specific polymer-trigger patterns identifiable from preclinical in vivo data

The central results block of the dissertation was provided by the structured processing of preclinical in vivo data. The organization of the 65 studies showed that the distribution of biodegradable, stimulus-responsive polymer carrier systems across different disease groups is not random. Recurrent associations emerged among trigger mechanisms, polymer families, polymer-chemical background, and disease categories that went beyond the level of individual formulation examples. The real outcome of this analysis, therefore, was not simply the summary of studies, but the demonstration that an implicit, disease-specific formulation logic is discernible in the preclinical in vivo data. [23]

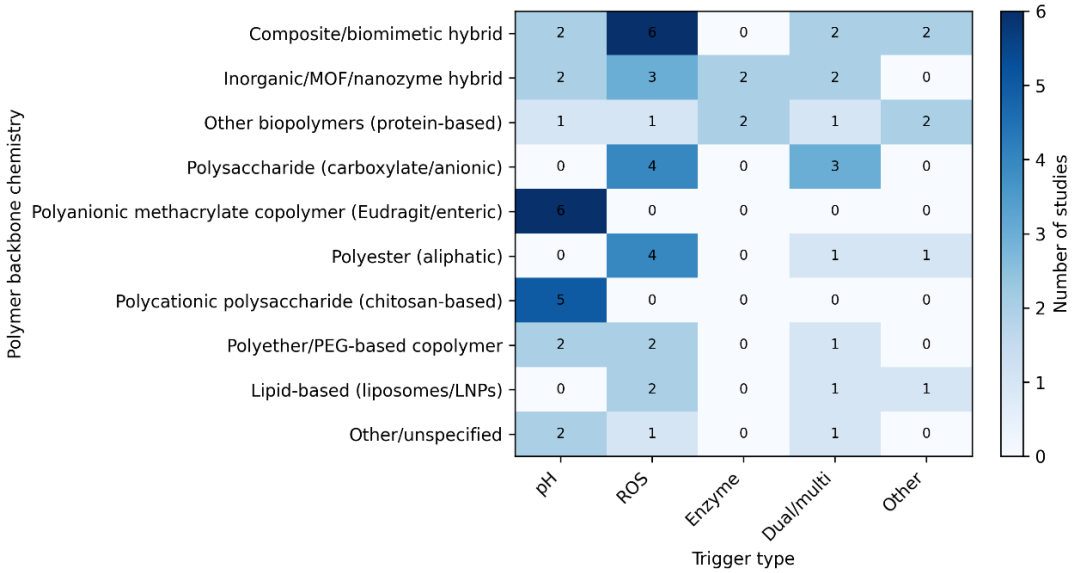

Figure 9. *Polymer backbone chemistry versus dominant trigger mechanism across the included studies (n=65). [23]*

One of the most important findings of the polymer study was that the pattern of stimulus responsiveness followed primarily polymer backbone chemistry rather than carrier format. Cross-tabulation of the 65 preclinical in vivo studies showed that polyanionic methacrylate copolymers, particularly Eudragit-type systems, appeared predominantly in pH-responsive settings; polycationic polysaccharides, mainly chitosan-based carriers, were likewise associated predominantly with pH-driven release; by contrast, anionic polysaccharides occurred more frequently in multi-trigger systems. Aliphatic polyesters, chiefly PLGA- and PLA-based platforms, were present across several trigger categories but showed recurrent occurrence particularly in ROS-based and combined mechanisms. Polyethers, including PEG derivatives and PEG-PPS systems, clustered strongly with ROS-activated platforms, whereas MOF- and nanozyme-containing hybrid systems appeared mainly in ROS-linked or combined trigger environments. Cross-linked polymer networks were found primarily in pH- and enzyme-sensitive systems. On this basis, one central scientific novelty of the polymer study was that trigger sensitivity followed the chemical structure of the polymer; that is, the response mechanism became interpretable not merely as a functional design choice, but as a formulation property linked to chemical structure. [23]

Overall, the aggregated results showed that inflammatory bowel diseases were dominated by systems associated with pH, ionic, and microbial trigger mechanisms [1-4]; joint and cartilage disorders by enzyme-sensitive, matrix-fitted platforms [13, 35]; cardiovascular and other oxidative-inflammatory models by ROS-/redox-responsive systems [15, 16]; and tumor settings by dual- and multi-trigger systems, often composite or hybrid in nature [12, 18, 19]. This four-part pattern may be regarded as the principal result of the dissertation because it directly shows a structured relationship between polymer selection and the dominant local trigger environment [23].

A key result of the polymer study was that the distribution of dominant trigger mechanisms did not follow polymer families randomly, but showed recurrent associations linked to polymer backbone chemistry [23]. Eudragit-, chitosan-, alginate-, and other polysaccharide-based systems were overrepresented in intestinal indications [5-7]; hyaluronic acid-, collagen-, and related matrix systems in joint models [36-38]; redox-sensitive polyethers and related platforms in oxidative environments [9]; and multi-component hybrid systems in tumor models [20, 39, 40]. Structured data extraction therefore revealed correspondences between polymer chemistry and the pathological environment.

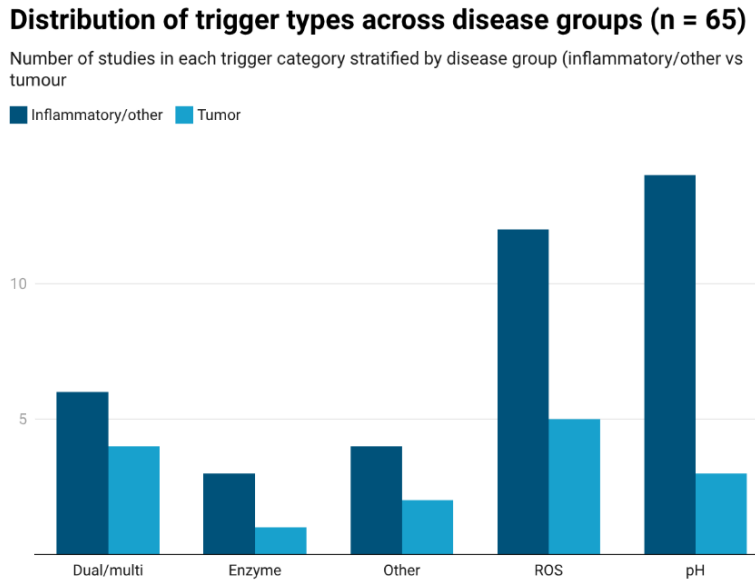

Figure 10. *Distribution of trigger types across disease groups in the 65 included studies. [23]*

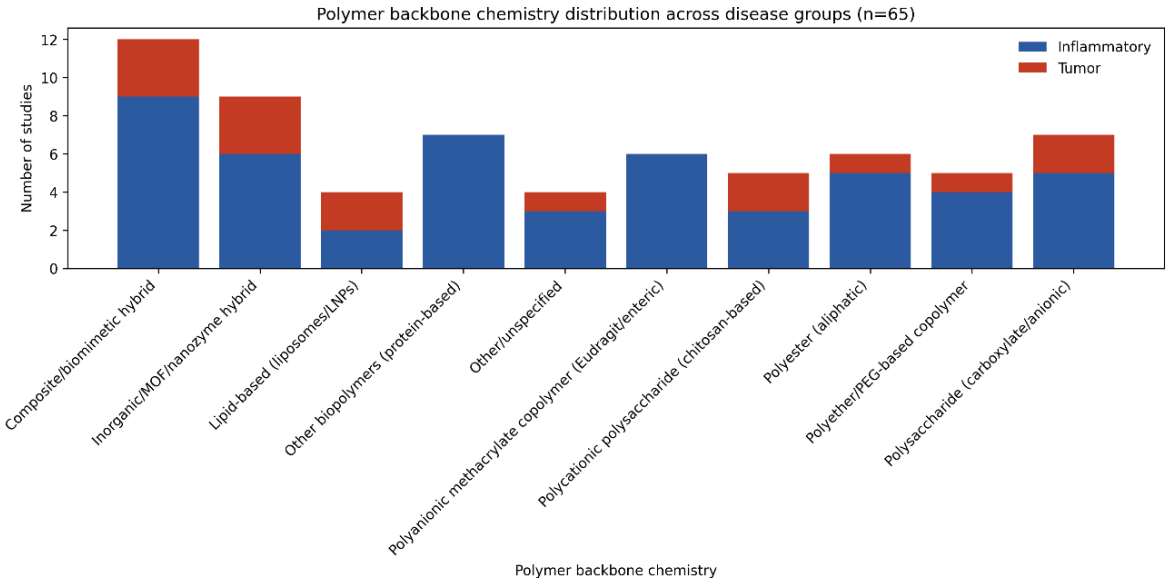

Figure 11. *Polymer chemistry distribution across disease groups in the 65 included studies. [23]*

Taken together, the two figures illustrate disease-specific differences in trigger distribution and polymer-chemical background, thereby visualizing one of the most important system-level claims of the entire dissertation.

4.4.1. Inflammatory bowel diseases

In intestinal inflammatory models, the carrier must remain sufficiently stable in the upper gastrointestinal tract and then release its payload in the colon under altered local pH and inflammatory conditions [1-4]. The recurrent success of these systems indicates that pH-responsive design is selected because it fits the intestinal disease environment, rather than merely because it is technically convenient.

Three recurrent intestinal formulation strategies were identifiable. Classical pH-dependent activation uses the gastrointestinal pH gradient to limit premature release [7, 42, 43, 46, 47]. Local retention is enhanced by mucoadhesive or bioadhesive components [41, 45]. Microbial or enzymatic activation exploits colon-specific biological activity [5, 6, 14]. Several platforms combined these mechanisms, showing that adaptation to the intestinal microenvironment often requires simultaneous optimization of more than one parameter [5, 6, 42].

Eudragit-based systems exploit ionization-dependent solubility for colon-targeted release [42, 47], while chitosan- and other polysaccharide-based carriers provide pH-sensitive, mucoadhesive, or microbially responsive behavior [5, 6, 43, 45]. These primary reports support the close relationship between polymer backbone chemistry and the dominant intestinal trigger mechanism.

Inflamed intestinal tissue simultaneously presents altered pH, oxidative stress, mucus changes, microbial dysbiosis, and local enzymatic activity. Accordingly, dual- and multi-trigger platforms and systems containing bioadhesive, antioxidant, or nanozyme components were prominent in the primary literature [5, 6, 12, 41-45]. The intestinal studies therefore provide strong evidence that carrier design is an adaptation to the local microenvironmental architecture of disease.

4.4.2. Joint and cartilage disorders

In joint and cartilage disorders, the dominant formulation challenges are local retention, adaptation to the damaged extracellular matrix, and release in a protease-rich inflammatory environment. Accordingly, enzyme-sensitive and matrix-associated systems, rather than enteric pH-responsive designs, were prominent [13, 35-38, 48, 49].

Hyaluronic acid-, collagen-, and related composite matrices play a special role because their chemical and biological properties support integration with damaged cartilage and the synovial environment [13, 36, 38, 49]. Biomimetic matrix design is therefore a formulation response to the structure of the target tissue.

Matrix metalloproteinase-responsive collagen-hyaluronic acid hydrogels directly couple degradation and release to protease activity in diseased tissue [13]. Other injectable or matrix-associated systems likewise exploit local inflammatory or oxidative features [35, 48]. Thus, carrier function is regulated by an active component of the pathological environment rather than by a nonspecific external trigger.

Local retention is an independent formulation priority in joint disease: performance depends on residence time in the intra-articular space, interaction with extracellular-matrix components, and behavior under synovial and mechanical conditions [35-38, 49]. Polymer selection is therefore a combined problem of trigger sensitivity and adaptation to biological architecture.

4.4.3. Cardiovascular and other oxidative-inflammatory conditions

ROS-/redox-responsive systems formed a distinct cluster in cardiovascular and other oxidative-inflammatory models [8, 31-34, 50]. In atherosclerosis, ROS-responsive and combined shear-stress/ROS-responsive platforms directly exploited plaque-associated oxidative and hemodynamic conditions [15-17].

In these models, ROS may act simultaneously as a damaging disease mediator, a marker of the local environment, and an activation signal for the carrier [8, 15-17, 31-34]. This convergence makes redox-sensitive polymers particularly suitable for cardiovascular applications.

The primary reports illustrate several redox-responsive design logics: cleavage or transformation of ROS-sensitive structures [8, 31, 33, 34], size-reducing nanoassemblies that improve plaque penetration [17], and biomimetic or RBC-hitchhiking systems that incorporate circulatory and shear conditions [15, 16]. Adaptation to the cardiovascular microenvironment is therefore multidimensional.

The earlier animal findings showed that pharmacological modification of myeloperoxidase activity was accompanied by structural changes in the aortic wall [22]. Together with contemporary ROS-responsive cardiovascular studies [8, 15-17, 31-34], this supports the interpretation that the oxidative and inflammatory microenvironment is an active factor shaping tissue structure, not merely a biochemical background phenomenon.

4.4.4. Tumor microenvironment

Tumor models showed overrepresentation of multi-stimulus, composite, and hybrid systems. pH-, ROS-, and enzyme-sensitive mechanisms were all represented, but many platforms combined several microenvironmental features [12, 18-20, 39, 40, 51-55]. This pattern distinguishes tumor formulations from the more dominant single-axis logic of several intestinal or joint systems.

Tumor microenvironments combine acidity, oxidative stress, heterogeneous perfusion, hypoxia, proteolytic activity, and immune alterations. Multi-trigger systems are therefore

an adaptation to biological heterogeneity rather than technological overdesign [12, 18-20, 40, 51-55].

Primary reports included redox/enzyme-responsive systems [12], metal-ion or MOF-containing hybrid platforms [18-20], and other composite carrier strategies [40, 51-55]. These examples show that tumor formulation often requires hybrid materials-science approaches beyond the logic of a single polymer family.

In several tumor platforms, the trigger mechanism supported not only release but also active remodeling of the microenvironment through chemodynamic, immunomodulatory, or combined antitumor effects [18-20, 51-55]. Thus, a microenvironment-based approach can regulate both the site and timing of release and the therapeutic mechanism itself.

The practical significance of pharmaceutical compounding adapted to individual therapeutic needs has long been recognized in pharmacy, which also points to the fact that the value of formulation cannot be judged exclusively on the basis of general technological parameters, but must also take into account the application environment and patient-specific needs. [56]

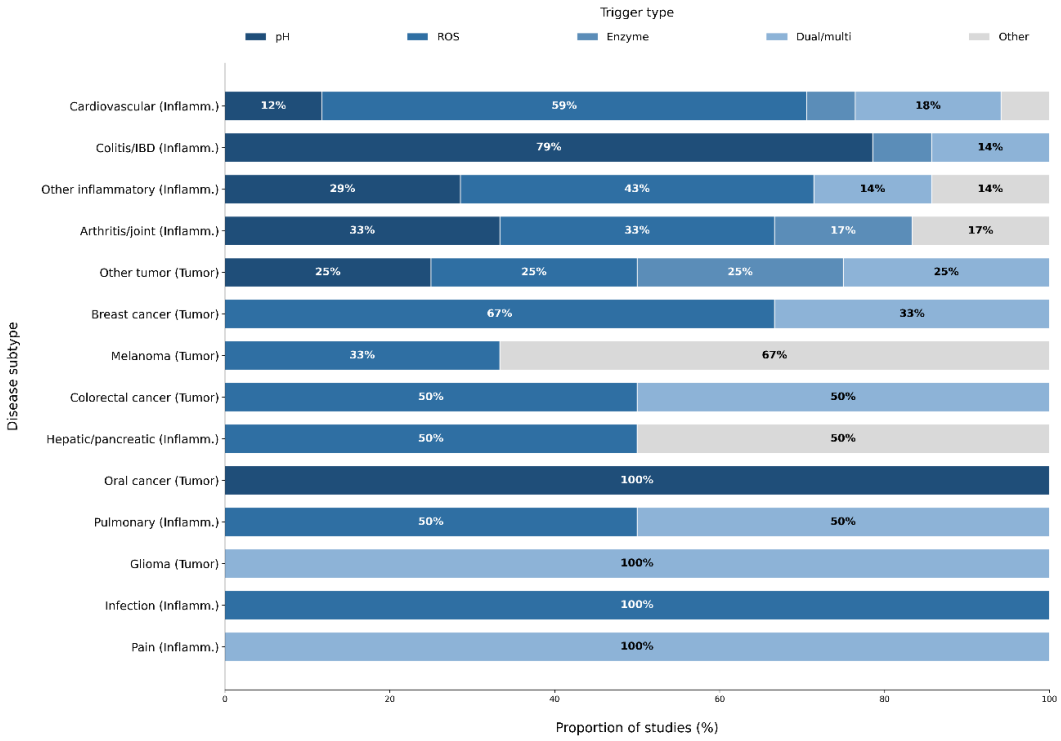

Figure 12. *Normalized trigger-type distribution across inflammatory and tumor disease subtypes [23]*

Figure 12 clearly illustrates that the trigger profiles of disease subcategories are not uniform: in inflammatory models, pH- and ROS-based mechanisms are prominent; in cardiovascular inflammatory conditions, ROS dominance is more pronounced; while in tumor subgroups, dual- and multi-trigger logic is more strongly emphasized.

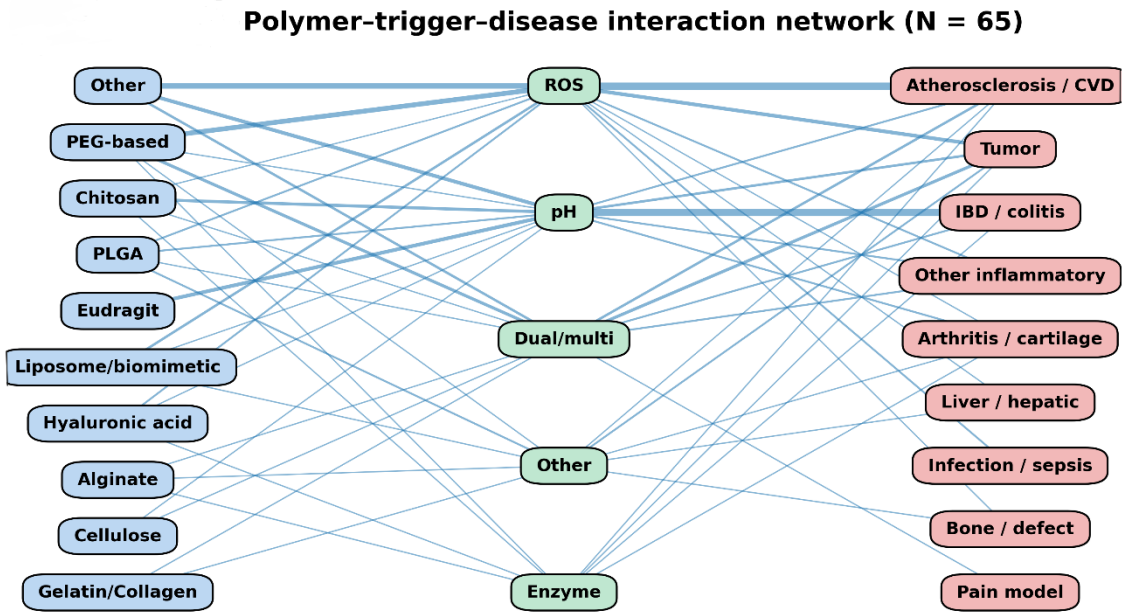

Figure 13. *Polymer-trigger-disease interaction network. [23]*

4.5. Summary of results

Earlier experimental, human, and animal observations, together with the structured processing of preclinical in vivo data, point in mutually compatible and mutually reinforcing directions. The performance of polymer carrier systems cannot be separated from the pathological environment in which they must function, and successful formulations observed in different indications are not distributed randomly among polymer families and trigger mechanisms. The most important result of Chapter 4 as a whole is therefore not a descriptive summary of individual carrier systems, but the demonstration that an implicit formulation structure underlies the preclinical in vivo data.

In inflammatory bowel diseases, systems fitted to pH, ionic, and microbial trigger mechanisms, mostly polysaccharide- and methacrylate-based, appeared recurrently. In joint and cartilage models, by contrast, enzyme-sensitive, biomimetic, and matrix-associated systems dominated, especially those based on hyaluronic acid and collagen. In cardiovascular and other oxidative-inflammatory indications, redox-responsive platforms, often combined with shear stress, came to the fore. In tumor settings, systems responding to multiple stimuli and fitted to a heterogeneous microenvironment, often composite or hybrid, became prominent. These differences are not merely thematic variations, but formulation patterns organized along dominant pathobiochemical driving forces. [1, 12, 13, 15, 18]

At the same time, the essence of the formulation guide emerging from the polymer study was not merely that certain triggers are associated with particular diseases, but that these triggers were recurrently linked to polymers of defined chemical structure. In inflammatory intestinal models, the dominance of ionizable methacrylates and polysaccharides indicated that protonation capacity, ionic interactions, and polyelectrolytic character confer a direct formulation advantage in pH- and ion-driven environments. In joint and cartilage indications, the recurrent role of hyaluronic acid- and collagen-based matrices, as well as enzyme-sensitive hydrogels, suggested that the logic of degradation and retention is here tied to the structural properties of matrix-like natural polymers. In oxidative disorders and atherosclerosis, the clustering of polyether-based, redox-sensitive, or hybrid systems indicated that oxidation-sensitive bond chemistry and ROS-induced structural transformation constitute the relevant chemical response motif. In the tumor environment, the overrepresentation of multicomponent composite architectures and MOF- or nanozyme-based hybrids suggested that the heterogeneous microenvironment requires more robust, multi-point formulation strategies than single-material systems. The genuine scientific result of the guide, therefore, was that it rendered polymer selection interpretable within the three-way relationship among chemical structure, dominant trigger, and disease-specific microenvironment. [23]

One of the most important results of Chapter 4 is the demonstration that earlier experimental, human, and animal observations, together with the disease-specific polymer-trigger patterns emerging from preclinical in vivo data, can be integrated into a unified scientific interpretative framework. The pH- and ion-dependent matrix behavior

observed in model systems, the human findings indicating alterations of the chemical environment, the animal evidence for the oxidative vascular microenvironment, and the disease-specific polymer-trigger clusters identifiable on the basis of 65 preclinical studies all converged into one coherent logic.

From this synthesis arises the most important scientific proposition of the dissertation: the performance of biodegradable polymers is not a universal property, but depends on their fit to the dominant pathological microenvironmental triggers. Polymer selection is therefore not merely a matter of materials science or technological routine, but a disease-calibrated decision in which the structural and functional characteristics of the carrier material must be judged in accordance with the local pathophysiological conditions of the target disease.

The most important outcome of the chapter is therefore not the ranking of individual systems, but the demonstration that sound polymer selection is underpinned by a reconstructable, disease-specific formulation logic. In this interpretation, the microenvironment is not the passive background of disease, but one of the most important organizing principles of formulation decisions. This recognition establishes the conceptual basis of disease-calibrated, microenvironment-based polymer selection.

5. Discussion

Based on the results presented in the dissertation, the relationship between the pathological microenvironment and the function of biodegradable polymer carrier systems cannot be regarded as incidental. Earlier experimental observations, our own human and animal findings, and the structured processing of contemporary preclinical literature all point in the same direction: the performance of a carrier system cannot be separated from the local biochemical and physicochemical environment in which release, retention, and therapeutic action must occur. The central claim of the dissertation is therefore that polymer selection is not merely a matter of materials science or pharmaceutical technology routine, but a disease-calibrated decision whose interpretation requires consideration of the dominant microenvironmental characteristics of the target disease. [23]

The scientific contribution of the dissertation lies in demonstrating that a reproducible, system-level relationship exists between the dominant microenvironmental characteristics associated with different disease types and the recurrently successful polymer systems. The central result of the work is therefore not a restatement of the existence of stimulus-responsive carrier systems, but the demonstration that a structured, disease-specific formulation logic can be discerned behind polymer selection in preclinical in vivo data. The work is therefore not a simple descriptive overview, but a synthesizing, relationship-seeking analysis that makes the implicit formulation logic of the preclinical literature visible. [24, 27]

One of the most important lessons of the earlier model-type experimental findings was that polymer matrices respond sensitively to the pH and ionic composition of the medium. At the time, this recognition appeared primarily as a physicochemical issue: proof that matrix response is not an autonomous property of the polymer, but the resultant of interactions between the polymer and the surrounding medium. In retrospect, however, this observation can already be regarded as an antecedent of a broader interpretative framework. If polymer responsiveness depends sensitively on environmental parameters even in model media, then it is reasonable to assume that the distinct pathological environments associated with different diseases also influence the in vivo behavior of carrier systems in different ways. From this perspective, the relationship between polymer

and medium is not a preliminary, neutral technological issue, but one of the foundations of later disease-specific applications.

The human findings reinforced this idea from another perspective. In porphyria cutanea tarda, deviations in whole-blood elemental composition and in the correlations among the elements suggested that the disease state is associated with a rearrangement in the organization of the internal chemical milieu. From the perspective of the present dissertation, the significance of this does not lie in the notion that a specific elemental change would directly designate a particular carrier system, but rather in the fact that it supports, through a human example, that the chemical characteristics of the pathological environment are not static and do not necessarily approximate the physiological state. This insight places the concept of the microenvironment into a broader, system-level context and reinforces that consideration of environmental conditions is not merely a matter of in vitro modeling, but part of biological reality. [21]

The animal experimental findings on the oxidative vascular environment can be interpreted in a similar way. Structural changes of the vascular wall associated with modification of myeloperoxidase activity indicated that oxidative and inflammatory states are not merely biochemical background phenomena, but also factors shaping tissue structure. From this perspective, the logic of later ROS-responsive cardiovascular carrier systems is readily understandable: these are not simply platforms that function in an oxidative environment, but carriers specifically tuned to a pathological state, transforming a defining biochemical characteristic of the disease into a therapeutic advantage. The relationship between our own animal experimental findings and contemporary redox-responsive platforms is therefore not a retrospective association, but a substantively consistent developmental line. [8, 9, 15-17, 22]

The structural interpretation of the vascular findings is further strengthened by the marked morphological parallel between the environment-dependent rearrangement observed in polymer matrices and the vessel wall distribution of atherosclerotic plaques. In the polymer system, water uptake, swelling, and particle movements do not occur homogeneously, but proceed in a front-like, spatially differentiated manner originating from surface-adjacent zones, while the inner regions show relative stability. In parallel, in the atherosclerotic aorta, plaques do not appear as a uniform coating, but as local,

spatially organized deposits. The comparison of these two phenomena suggests that in both cases the structural response is organized in relation to local microenvironmental conditions. From this perspective, pathological vascular wall remodeling may be interpreted not only as a disease process, but also as a natural organizational pattern that may offer relevant insights for the design of microenvironment-adapted polymer systems. In the future, this concept may contribute to the development of polymer-based platforms optimized specifically for vascular applications through local adhesion, structural adaptation, and controlled drug release.

The most important result of processing the preclinical in vivo data was that formulation solutions that had previously appeared as scattered examples became interpretable as structured patterns. On the basis of the preclinical reports examined, inflammatory bowel diseases were characterized primarily by polysaccharide- and methacrylate-based systems fitted to pH, ionic, and microbial activation; joint and cartilage disorders by enzyme-sensitive matrices, especially hyaluronic acid-based and collagen-associated ones; atherosclerosis and other oxidative-inflammatory indications by ROS- and shear stress-responsive platforms; and tumor settings by composite and hybrid systems responding to multiple stimuli and adapted to a heterogeneous microenvironment. This pattern suggests that successful carrier systems are not distributed randomly across diseases, but cluster along dominant pathobiochemical trigger mechanisms. [1-7, 12, 13, 18-20, 35, 36]

The example of inflammatory bowel diseases illustrates this relationship particularly well. In this indication, the goal is not simply to deliver the active agent to the gastrointestinal tract, but to ensure release in the appropriate segment, fitted to the inflamed environment, often by exploiting colon-specific pH conditions, mucosal binding, or microbial activity. The combination of mucoadhesion, pH-dependent activation, colon-specific microbial cleavage, and local retention indicates that successful intestinal platforms are based on multidimensional environmental fit. The recurrent appearance of such systems strongly suggests that, here, it is indeed the characteristics of the local environment that govern the formulation logic. [14, 41-46]

In joint and cartilage disorders, the pattern is different in nature, but equally consistent. Here, the protease-rich and structurally damaged extracellular environment renders the

use of enzyme-sensitive, matrix-associated, and biomimetic systems intelligible. The recurrent dominance of hyaluronic acid and collagen derives not simply from their being favorable biomaterials, but from their fit to the biological architecture of the target environment. The combination of local retention, intra-articular stability, and protease-dependent degradation shows that, in this disease group, polymer selection is not merely a matter of trigger sensitivity, but also of compatibility with tissue structure. [37, 38, 48, 49]

In cardiovascular indications, the situation is again different, yet interpretable according to the same principle. Here, the expansion of redox-sensitive platforms corresponds to the fact that local ROS excess is not only a damaging factor, but also a potential release trigger. In this setting, ROS is both a component of pathology and a key to therapeutic activation. Shear stress/ROS-dual systems, biomimetic micelles, RBC-hitchhiking platforms, and size-reducible nanoassemblies all indicate that, in cardiovascular carrier systems, the environmental trigger and the therapeutic strategy are often connected to the same pathobiochemical axis. [31, 33, 34, 50]

The predominance of multi-trigger and hybrid systems in the tumor microenvironment can be understood from the fact that the tumor milieu itself is heterogeneous. Because lower pH, oxidative stress, hypoxia, proteolytic activity, perfusion irregularities, and immunological components occur in spatially and temporally varying combinations, a single trigger is often insufficient to ensure adequately robust activation. Dual- and multi-trigger systems, as well as platforms containing composite, metal-ion, or MOF components, therefore reflect not a disorderly technological diversity, but appropriate adaptation to the heterogeneous tumor microenvironment. The tumor block provides particularly strong evidence that the more complex the pathological environment, the more complex the formulation solutions that recurrently prove successful. [40, 51-55]

A key proposition of the dissertation is therefore that the performance of biodegradable polymers is not a universal property. The same system may have different value in different biological environments, and formulation success depends to a great extent on how well the structural and functional characteristics of the carrier correspond to the dominant microenvironmental characteristics of the target disease. From this perspective, polymer selection may be reinterpreted as a disease-calibrated decision. This approach

does not negate the importance of materials-related, technological, or manufacturability considerations, but complements them with the criterion of pathophysiological fit. [23]

Beyond the major disease groups, additional examples from contemporary preclinical literature also reinforce that formulation rationality in other indications likewise rests on adaptation to the microenvironment created by disease. This is clearly seen in maxillofacial infection, diabetic wound healing, luminal diagnostic approaches in inflammatory bowel disease, oral 5-FU delivery, pneumonia, lupus nephritis, intervertebral disc degeneration, tuberculous granuloma, periodontitis, and local, timed, or sequential drug-delivery systems, in which local oxidative, inflammatory, infectious, or regenerative environmental parameters directly determine the logic of carrier-system design. [57-72]

Similarly, a further range of cardiovascular, neurovascular, and regenerative examples shows that pathological microenvironments of atherosclerotic, ischemic, endothelial, or valvular origin are not merely background conditions, but primary determinants of activation, targeting, tissue retention, and functional effect. Stroke models, myocardial ischemia-reperfusion injury, endothelial damage, bioprosthetic and tissue-replacement cardiovascular systems, and different forms of atherosclerosis all suggest that the fit between microenvironmental trigger and polymer selection may be interpreted as a generalizable formulation principle. [73-84]

This interpretative approach nevertheless has clear limitations. The heterogeneity of preclinical models, differing endpoints, different routes of administration, and the variable numerical detail of the reports did not allow direct quantitative comparison across all systems. The strength of the work therefore lies not in establishing pooled effect sizes, but in recognizing structural relationships. The central question was not which individual system is the best, but which types of polymer functions prove recurrently adequate for which kinds of microenvironmental challenges. In this sense, the present dissertation provides a formulation map rather than a classical efficacy ranking. [25, 26, 28]

Based on the above findings, it appears justified to conclude that, in the future development of biodegradable polymer carrier systems, more detailed consideration of the pathological microenvironment of diseases may become not merely a theoretical

advantage, but a foundation for more rational formulation decision-making. Interpreting the fit between polymer and disease in this way points toward more personalized drug-carrier development. The basis of this view is that the focus of formulation shifts not to the general physiological environment of the affected organ, but to the characteristic pathological microenvironment created by the disease. Within this framework, the microenvironment is not a passive background, but one of the primary organizing principles of polymer selection.

6. Conclusions

Based on the findings of the dissertation, it can be concluded that the interpretation and selection of biodegradable polymer-based carrier systems cannot be confined to conventional material and technological considerations alone. The performance of these delivery systems is substantially determined by the pathological microenvironment in which drug release, local retention, target-site delivery, and therapeutic action must occur. Accordingly, pH, redox status, enzymatic activity, ionic conditions, microbial influences, and, in certain indications, mechanical and flow-related factors emerge as functionally relevant parameters that directly shape carrier performance.

The central conclusion of the work is that the focus of formulation shifts away from the general physiological environment of the affected organ toward the characteristic pathological microenvironment created or modified by the disease. Within this interpretive framework, the microenvironment is not a passive background, but one of the primary organizing principles of polymer selection. Consequently, the fit between a carrier system and a target disease should not be judged on the basis of generic technological advantages, but rather on its correspondence to the dominant local pathobiochemical features.

One of the most important system-level conclusions of the dissertation is that distinct disease groups are associated with different, yet consistently recurring polymer-trigger patterns. This makes it possible to interpret polymer selection not as a collection of isolated formulation examples, but as a reconstructable decision logic.

Earlier experimental results showed that the behavior of polymer matrices responds sensitively to the pH and ionic properties of the surrounding medium, and that even minor compositional modifications may lead to substantial differences in swelling, volume change, and release characteristics. In the context of the present dissertation, the significance of these observations lies in the fact that they demonstrated that the behavior of a carrier system cannot be described solely on the basis of material composition, but must always be interpreted as part of its interaction with the surrounding medium. This recognition constituted the experimental precursor of the later system-level perspective

in which the microenvironment is treated not merely as a technological background, but as an organizing principle of formulation.

The human findings enabled a further important conclusion. The differences in elemental composition and the altered correlation patterns observed in porphyria cutanea tarda indicated that the pathological state may be associated with an organized rearrangement of the internal chemical milieu. From this perspective, the concept of the microenvironment cannot be restricted exclusively to the local cellular environment or the anatomical milieu of a given organ, but may also be interpreted in a broader biological context. The human line of evidence therefore supports the conclusion that the chemical milieu associated with disease is not static, and that its deviation from the physiological reference state may also be relevant to the behavior of drug carrier systems.

The animal experimental findings likewise suggest that the oxidative and inflammatory microenvironment is not merely a biochemical background phenomenon, but an active factor shaping tissue structure. The vascular structural changes associated with altered myeloperoxidase activity indicate that redox status may function as a key organizing element of pathology [22]. This conclusion is of particular importance for the interpretation of later ROS-responsive carrier systems, as it confirms that platforms adapted to oxidative conditions are not artificially imposed technological constructs, but rational formulation solutions built upon dominant local features of the disease.

The central system-level conclusion of the dissertation emerged from the systematic analysis of the contemporary preclinical literature. Based on the evaluation of 65 in vivo studies, it was demonstrated that successful stimulus-responsive biodegradable carrier systems are not distributed randomly across disease groups. Specific trigger mechanisms and polymer families recur in association with particular indications, suggesting that an implicit formulation structure underlies the preclinical literature. One of the most important scientific findings of the dissertation is the elucidation of this hidden structure.

In inflammatory bowel diseases, the main conclusion is that successful delivery systems are predominantly platforms adapted to the specific features of the inflamed intestinal environment. The pH gradient, ionic conditions, alterations of the mucus layer, microbial activity, and local inflammation together create a milieu in which pH-responsive, ionizable, mucoadhesive, or microbially activated systems prove consistently

advantageous. The principal conclusion in this disease group is therefore that formulation success is closely linked to multidimensional adaptation to the colon-specific inflamed microenvironment.

In joint and cartilage disorders, a different but equally consistent formulation logic emerged. In this disease group, the dominant factor is not the classical pH or ionic trigger, but adaptation to a protease-rich environment characterized by structural damage and altered extracellular matrix organization. The recurrent presence of hyaluronic acid-, collagen-, and other biomimetic matrices, as well as enzyme-sensitive hydrogels, suggests that polymer selection in this context is not merely a question of release-trigger sensitivity, but also of compatibility with biological architecture. It follows that in cartilage and joint-related indications, successful formulation is fundamentally based on adaptation to the structural and degradative properties of the tissue environment.

In cardiovascular and other oxidative-inflammatory conditions, the most important conclusion is that redox status is not merely a marker of disease, but also an active organizing factor in carrier system performance. The recurring presence of ROS-responsive, shear stress-associated, biomimetic, or structurally transforming platforms indicates that polymer selection in this disease group is aligned with the dominant oxidative and hemodynamic features of the pathology. The success of redox-sensitive systems is thus a direct consequence of the fact that the release trigger coincides with one of the fundamental pathobiochemical characteristics of the disease.

The findings related to the tumor microenvironment indicate that the more complex and heterogeneous the pathological milieu is, the more important dual- and multi-trigger systems, as well as composite and hybrid platforms, become. In the tumor microenvironment, the simultaneous presence of acidic pH, oxidative stress, hypoxia, proteolytic activity, perfusion heterogeneity, and immunological factors often renders single-trigger systems insufficient. Accordingly, those platforms came to the forefront in tumor models that are capable of adapting to the heterogeneous environment through multiple points of interaction. The main conclusion here is that the complexity of the tumor microenvironment is mirrored in the complexity of the formulation solutions.

Recurrent polymer-chemical motifs underlie these disease-specific trigger preferences. In pH- and ion-dependent intestinal systems, ionizable carboxylate and amino groups

became decisive; in redox-driven vascular and tumor systems, oxidation-sensitive bonds and polyether structural motifs were prominent; whereas in joint and cartilage disorders, enzymatically cleavable, matrix-compatible natural polymer networks became characteristic. Polymer selection should therefore be interpreted not only according to material family or carrier form, but also according to the fit between the chemical responsiveness of the polymer and the dominant pathological microenvironmental driving force.

One of the most important system-level conclusions of the dissertation, therefore, is that distinct disease groups are associated with different, yet consistently recurring polymer-trigger patterns. The morphological parallel between the spatial rearrangement of polymer matrices and pathological tissue organization suggests that pathological structural patterns observed in nature may serve not only as interpretive models, but also as starting points for the development of microenvironment-calibrated polymer systems with local protective, adhesive, or controlled drug-release properties. This recognition makes it possible to interpret polymer selection not merely as a collection of isolated formulation examples, but as a reconstructable decision logic. The true novelty of the work lies precisely here: it does not merely demonstrate that stimulus-responsive systems exist, but shows that a system-level relationship can be identified between the disease-transformed microenvironment and the polymer solutions that repeatedly prove successful.

A further important conclusion of the dissertation is that earlier experimental, human, and animal observations, together with the disease-specific polymer-trigger patterns emerging from preclinical in vivo data, can be integrated into a coherent and unified interpretive scheme. The starting point of this scheme is the experimental recognition of the polymer-medium relationship; its biological foundation is provided by the human and animal findings; and its system-level extension is represented by the identification of polymer-trigger patterns associated with disease types. Accordingly, the principal conceptual outcome of the dissertation is the formulation of disease-calibrated, microenvironment-based polymer selection as both an interpretive and a decision-making framework.

At the same time, an important conclusion is that this perspective does not replace classical pharmaceutical technological considerations. Biocompatibility, biodegradability, manufacturability, stability, dosage feasibility, and therapeutic safety

remain fundamentally important. Rather, the conclusion of the present work is that alongside these considerations, an additional, higher-level organizing principle is required: the assessment of the extent to which a given carrier system corresponds to the dominant pathological microenvironmental features of the target disease. Pathophysiological fit thus emerges as a new dimension of formulation rationality.

The presented results also imply that, in future drug carrier development, more detailed characterization of the local microenvironmental profile of diseases may be of particular importance. The more precisely it can be determined in which local parameters a given disease differs from the physiological state, the more rationally it can be judged which polymer families, response mechanisms, and carrier-system types are suitable for achieving the therapeutic goal. From this perspective, the present dissertation offers not a closed system, but a conceptual framework that can be further developed.

Overall, it may therefore be concluded that the concept of polymer selection adapted to the pathological microenvironment created or modified by disease is suitable for placing the interpretation of biodegradable drug carrier systems on new foundations. Accordingly, the most important final conclusion of the work is that the microenvironment is not a passive background of formulation, but one of its primary organizing principles. This perspective may also provide a relevant starting point for future drug carrier systems that are more personalized, more precisely targeted in pathophysiological terms, and more rationally designed.

7. Summary

The central proposition of the dissertation is that, in the design and selection of biodegradable polymer carrier systems, the primary organizing principle should be sought not in the general environment of the target organ, but in the disease-specific pathological microenvironment.

- Based on earlier experimental, human, and animal findings, together with the analysis of 65 preclinical in vivo studies, it was demonstrated that the performance of polymer systems is not determined by any universal material property, but by the extent to which they fit the dominant local pathobiochemical drivers of disease.
- Successful carrier systems were associated not with isolated formulation solutions, but with recurring, disease-specific polymer-trigger patterns.
- In inflammatory intestinal models, ionizable polysaccharide- and methacrylate-based systems predominated, whereas in joint and cartilage disorders enzyme-sensitive, hyaluronic acid-based matrices proved most characteristic.
- In oxidative-inflammatory vascular and tumor microenvironments, redox-sensitive polyethers and hybrid platforms recurred, while in the heterogeneous tumor microenvironment multi-responsive composite systems were particularly prominent.
- A morphological parallel was identified between the spatial rearrangement of polymer matrices and the structural organization of atherosclerotic plaques, suggesting that pathological tissue patterns may, in the future, serve as models for the development of locally protective, adhesive, or controlled-release polymer systems.
- Taken together, these findings indicate that polymer selection should be interpreted not as a routine technological choice, but as a disease-calibrated decision grounded in both pathophysiological and chemical considerations, thereby enabling more rational and more targeted formulation strategies.

8. References

1. Cai, X.; Wang, X.; He, M.; Wang, Y.; Lan, M.; Zhao, Y.; Gao, F. Colon-targeted delivery of tacrolimus using pH-responsive polymeric nanoparticles for murine colitis therapy. Int. J. Pharm. 2021, 606, 120836. https://doi.org/10.1016/j.ijpharm.2021.120836.

2. Liu, H.; Cai, Z.; Wang, F.; Hong, L.; Deng, L.; Zhong, J.; Wang, Z.; Cui, W. Colon-Targeted Adhesive Hydrogel Microsphere for Regulation of Gut Immunity and Flora. Adv. Sci. 2021, 8, e2101619. https://doi.org/10.1002/advs.202101619.

3. Feng, J.; Wang, Y.; Lv, Y.; Fang, S.; Ren, M.; Yao, M.; Lan, M.; Zhao, Y.; Gao, F. A pH-Responsive and Colitis-Targeted Nanoparticle Loaded with Shikonin for the Oral Treatment of Inflammatory Bowel Disease in Mice. Mol. Pharm. 2022, 19, 4157-4170. https://doi.org/10.1021/acs.molpharmaceut.2c00550.

4. Ali, A.S.; Altayari, A.A.; Khan, L.M.; Alharthi, S.E.; Ahmed, O.A.; El-Shitany, N.A.; Ali, S.S.; Saadah, O.I. Colon-targeted therapy of tacrolimus (FK506) in the treatment of experimentally induced colitis. Pharmacology 2020, 105, 541-549.

https://doi.org/10.1159/000505101.

5. Lin, Y.; Fang, S.; Chen, X.; Li, X.; Zhao, X.; Wang, Y.; Lu, J.; Ji, Q.; Zheng, S.; Zou, J.; Qi C.; Gao F. Pectinase-responsive nanoplatform for oral targeted delivery of 5-aminosalicylic acid and zinc coordination-driven nanozyme in inflammatory bowel disease treatment. Int. J. Pharm. X 2025, 10, 100362.

https://doi.org/10.1016/j.ijpx.2025.100362.

6. Zeeshan, M.; Ain, Q.U.; Weigmann, B.; Story, D.; Smith, B.R.; Ali, H. Dual pH and microbial-sensitive galactosylated polymeric nanocargoes for multi-level targeting to combat ulcerative colitis. Asian J. Pharm. Sci. 2023, 18, 100831.

7. Zhao, Y.; Xu, C.; Liu, Q.; Lei, X.; Deng, L.; Wang, F.; Yang, J. pH-responsive interface conversion efficient oral drug delivery platform for alleviating inflammatory bowel disease. Front. Chem. 2024, 12, 1365880. https://doi.org/10.3389/fchem.2024.1365880.

8. Poole, K.M.; Nelson, C.E.; Joshi, R.V.; Martin, J.R.; Gupta, M.K.; Haws, S.C.; Kavanaugh, T.E.; Skala, M.C.; Duvall, C.L. ROS-responsive microspheres for on-

demand antioxidant therapy in a model of diabetic peripheral arterial disease. Biomaterials 2015, 41, 166-175. https://doi.org/10.1016/j.biomaterials.2014.11.016.

9. Chen, R.; Xia, Y.; Ma, Z.; Ke, Y.; Shi, Q.; Ataullakhanov, F.I.; Panteleev, M. H2O2-responsive polymeric micelles of biodegradable aliphatic poly(carbonate)s as promising therapeutic agents for inflammatory diseases. Macromol. Biosci. 2024, 24, e2300409. https://doi.org/10.1002/mabi.202300409.

10. Sun, X.; Yu, K.; Zhou, Y.; Dong, S.; Hu, W.; Sun, Y.; Li, Y.; Xie, J.; Lee, R.J.; Sun, F.; Ma Y.; Wang S.; Kim BYS.; Wang Y.; Yang Z.; Jiang W.; Li Y.; Teng L. Self-assembled pH-sensitive polymeric nanoparticles for the inflammation-targeted delivery of Cu/Zn-superoxide dismutase. ACS Appl. Mater. Interfaces 2021, 13, 18152-18164.

https://doi.org/10.1021/acsami.1c03589.

11. Wang, Q.Y.; Xu, Y.S.; Zhang, N.X.; Dong, Z.P.; Zhao, B.N.; Liu, L.C.; Lu, T.; Wang, Y. Phenylboronic ester-modified anionic micelles for ROS-stimuli response in HeLa cells. Drug Deliv. 2020, 27, 681-690. https://doi.org/10.1080/10717544.2020.1748761.

12. Zhang, X.; Lian, R.; Fan, B.; Meng, L.; Zhang, P.; Zhang, Y.; Sun, W. ROS/enzyme dual-responsive drug delivery system for targeted colorectal cancer therapy: Synergistic chemotherapy, anti-inflammatory, and gut microbiota modulation. Pharmaceutics 2025, 17, 940. https://doi.org/10.3390/pharmaceutics17070940.

13. Yi, X.; Xu, Z.; Liu, Q.; Zhou, H.; Yuan, L.; Li, D.; Zhao, L.; Mu, C.; Ge, L. Matrix metalloproteinase-responsive collagen-oxidized hyaluronic acid injectable hydrogels for osteoarthritic therapy. Biomater. Adv. 2022, 137, 212804.

https://doi.org/10.1016/j.bioadv.2022.212804.

14. Xie, X.; Wang, Y.; Deng, B.; Blatchley, M.R.; Lan, D.; Xie, Y.; Lei, M.; Liu, N.; Xu, F.; Wei, Z. Matrix metalloproteinase-responsive hydrogels with tunable retention for on-demand therapy of inflammatory bowel disease. Acta Biomater. 2024, 186, 354-368. https://doi.org/10.1016/j.actbio.2024.07.054.

15. Shen, M.; Li, H.; Yao, S.; Wu, X.; Liu, S.; Yang, Q.; Zhang, Y.; Du, J.; Qi, S.; Li, Y. Shear stress- and ROS-responsive biomimetic micelles for atherosclerosis via ROS

consumption. Mater. Sci. Eng. C Mater. Biol. Appl. 2021, 126, 112164. https://doi.org/10.1016/j.msec.2021.112164.

16. Shen, M.; Jiang, H.; Zhao, Y.; Wu, L.; Yang, H.; Yao, Y.; Meng, H.; Yang, Q.; Liu, L.; Li, Y. Shear stress- and ROS dual-responsive RBC-hitchhiking nanoparticles for atherosclerosis therapy. ACS Appl. Mater. Interfaces 2023, 15, 43374-43386. https://doi.org/10.1021/acsami.3c07371.

17. He, J.; Zhang, W.; Zhou, X.; Xu, F.; Zou, J.; Zhang, Q.; Zhao, Y.; He, H.; Yang, H.; Liu, J. Reactive oxygen species (ROS)-responsive size-reducible nanoassemblies for deeper atherosclerotic plaque penetration and enhanced macrophage-targeted drug delivery. Bioact. Mater. 2022, 19, 115-126.

https://doi.org/10.1016/j.bioactmat.2022.03.041.

18. Cheng, F.; Wang, S.; Zheng, H.; Yang, S.; Zhou, L.; Liu, K.; Zhang, Q.; Zhang, H. Cu-doped cerium oxide-based nanomedicine for tumor microenvironment-stimulative chemo-chemodynamic therapy with minimal side effects. Colloids Surf. B Biointerfaces 2021, 205, 111878. https://doi.org/10.1016/j.colsurfb.2021.111878.

19. Zheng, S.; Guo, Y.; Han, Q.; Peng, X.; Sheng, R.; Liu, S.; Li, Z. STING agonists and PI3Kγ inhibitor co-loaded ferric ion-punicalagin networks for comprehensive cancer therapy. Int. J. Biol. Macromol. 2024, 282, 136776. https://doi.org/10.1016/j.ijbiomac.2024.136776.

20. Zhang, L.; Lu, K.; Cao, Y.; Cui, L.; Li, X.; Guo, P.; Fu, H.; Zhu, C.H. An injectable dual-layer chitosan-alginate nano-hydrogel incorporated Cu(I)-isopolymolybdate-based metal-organic framework tailored for three-in-one anti-cancer nanotherapy. Int. J. Biol. Macromol. 2024, 282, 137381. https://doi.org/10.1016/j.ijbiomac.2024.137381.

21. Dinya, M.; Székely, E.; Szentmihályi, K.; Tasnádi, G.; Blázovics, A. Major and trace elements in whole blood of phlebotomized patients with porphyria cutanea tarda. J. Trace Elem. Med. Biol. 2005, 19, 217-220. https://doi.org/10.1016/j.jtemb.2005.07.011.

22. Békési G, Heinle H, Kakucs R, Pázmány T, Szombath D, Dinya M, Tulassay Z, Fehér J, Rácz K, Székács B, Riss E, Farkas A, Gódor F, Illyés G. Effect of inhibitors of

myeloperoxidase on the development of aortic atherosclerosis in an animal model. Exp Gerontol. 2005 Mar;40(3):199-208. doi: 10.1016/j.exger.2004.12.004.

23. Dinya, M.; Dinya, E.; Mórotz, G.M. From Pathology to Formulation: Designing Biodegradable Polymers for Personalized Drug Delivery. Pharmaceutics 2026, 18, 330. https://doi.org/10.3390/pharmaceutics18030330.

24. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; Chou R.; Glanville J.; Grimshaw JM.; Hróbjartsson A.; Lalu MM.; Li T.; Loder EW.; Mayo-Wilson E.; McDonald S.; McGuinness LA.; Stewart LA.; Thomas J.; Tricco AC.; Welch VA.; Whiting P.; Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021, 372, n71. https://doi.org/10.1136/bmj.n71.

25. Hooijmans, C.R.; Rovers, M.M.; de Vries, R.B.; Leenaars, M.; Ritskes-Hoitinga, M.; Langendam, M.W. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 2014, 14, 43. https://doi.org/10.1186/1471-2288-14-43.

26. Higgins, J.P.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analyses. BMJ 2003, 327, 557-560. https://doi.org/10.1136/bmj.327.7414.557.

27. McKenzie, J.E.; Brennan, S.E.; Ryan, R.E. Synthesizing and presenting findings using other methods. In Cochrane Handbook for Systematic Reviews of Interventions; Version 6.3; Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A., Eds.; Cochrane: London, UK, 2022.

28. Egger, M.; Davey Smith, G.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997, 315, 629-634. https://doi.org/10.1136/bmj.315.7109.629.

29. Dinya, M.; Antal, I. Új lehetőségek a gyógyszertechnológiában: intelligens hidrogélek. Gyógyszerészet 2002, 46(2), 67-71.

30. Antal, I.; Dinya, M. Intelligens polimerek alkalmazása a gyógyszer-technológiában. II. A hatóanyagleadás szabályozására alkalmas mechanizmusok. Gyógyszerészet 2002, 46(3), 141-145.

31. Gui, L.; Chen, Y.; Diao, Y.; Chen, Z.; Duan, J.; Liang, X.; Li, H.; Liu, K.; Miao, Y.; Gao, Q.; Li Z.; Yang J.; Li Y. ROS-responsive nanoparticle-mediated delivery of CYP2J2 gene for therapeutic angiogenesis in severe hindlimb ischemia. Mater. Today Bio 2021, 13, 100192. https://doi.org/10.1016/j.mtbio.2021.100192.

32. Wang, L.; Peng, Y.; Song, L.; Xia, D.; Li, C.; Li, Z.; Li, Q.; Yu, A.; Lu, C.; Wang, Y. Colchicine-containing nanoparticles attenuate acute myocardial infarction injury by inhibiting inflammation. Cardiovasc. Drugs Ther. 2022, 36, 1075-1089. https://doi.org/10.1007/s10557-021-07239-2.

33. Yang, H.; Guo, M.; Guan, Q.; Zhang, L.; Liu, M.; Li, H.; Qiao, G.; Yang, Q.; Shen, M.; Li, Y. ROS-responsive simvastatin nano-prodrug based on tertiary amine-oxide zwitterionic polymer for atherosclerotic therapy. J. Nanobiotechnol. 2025, 23, 176. https://doi.org/10.1186/s12951-025-03232-1. Erratum in J. Nanobiotechnol. 2025, 23, 277. https://doi.org/10.1186/s12951-025-03359-1.

34. Zhen, P.; Jiang, Q.; Yu, F.; Xu, X.; Wei, Q.; Liu, X.; Sun, X.; Liang, G.; Tong, J. ROS-differentiated release of Apelin-13 from hydrogel comprehensively treats myocardial ischemia-reperfusion injury. J. Control Release 2025, 379, 609-620. https://doi.org/10.1016/j.jconrel.2025.01.039. PMID: 39842726.

35. Li, H.; Lyu, Y.; Wang, R.; Yu, H.; Lin, M.; Li, Z.; Zhong, Y.; Sheng, P.; Zhang, K.; Liao, W.; Bian L. Supramolecular drug-laden hydrogel based on structural tautomerization enhances drug delivery for rheumatoid arthritis treatment. Bioact. Mater. 2025, 53, 495-506. https://doi.org/10.1016/j.bioactmat.2025.07.039.

36. Phan, V.H.G.; Murugesan, M.; Nguyen, P.P.T.; Luu, C.H.; Le, N.H.; Nguyen, H.T.; Manivasagan, P.; Jang, E.S.; Li, Y.; Thambi, T. Biomimetic injectable hydrogel based on silk fibroin/hyaluronic acid embedded with methylprednisolone for cartilage regeneration. Colloids Surf. B Biointerfaces 2022, 219, 112859. https://doi.org/10.1016/j.colsurfb.2022.112859.

37. Wang, Y.; Zhang, D.; Jia, M.; Zheng, X.; Liu, Y.; Wang, C.; Lei, F.; Niu, H.; Li, C. ZIF-8 nanoparticles coated with macrophage-derived microvesicles for sustained, targeted delivery of dexamethasone to arthritic joints. J. Drug Target. 2022, 30, 1006-1016. https://doi.org/10.1080/1061186X.2022.2077949.

38. Yang, Z.; Cao, F.; Li, H.; He, S.; Zhao, T.; Deng, H.; Li, J.; Sun, Z.; Hao, C.; Xu, J.; Guo Q.; Liu S.; Guo W. Microenvironmentally optimized 3D-printed TGFβ-functionalized scaffolds facilitate endogenous cartilage regeneration in sheep. Acta Biomater. 2022, 150, 181-198. https://doi.org/10.1016/j.actbio.2022.07.029.

39. Poonia, N.; Lather, V.; Narang, J.K.; Beg, S.; Pandita, D. Resveratrol-loaded folate-targeted lipoprotein-mimetic nanoparticles with improved cytotoxicity, antioxidant activity and pharmacokinetic profile. Mater. Sci. Eng. C Mater. Biol. Appl. 2020, 114, 111016. https://doi.org/10.1016/j.msec.2020.111016.

40. Hsu, C.Y.; Lin, J.; Wei, M.F.; Chen, L.H.; Liang, H.T.; Lin, F.H. Local delivery of carboplatin-loaded hydrogel and calcium carbonate enables two-stage drug release for limited-dose radiation to eliminate mouse malignant glioma. Biomaterials 2025, 312, 122746. https://doi.org/10.1016/j.biomaterials.2024.122746.

41. Wang, D.; Sun, M.; Zhang, Y.; Chen, Z.; Zang, S.; Li, G.; Li, G.; Clark, A.R.; Huang, J.; Si, L. Enhanced therapeutic efficacy of a novel colon-specific nanosystem loading emodin on DSS-induced experimental colitis. Phytomedicine 2020, 78, 153293. https://doi.org/10.1016/j.phymed.2020.153293.

42. Maleki, H.; Doostan, M.; Farzaei, M.H.; Seifi, P.; Miraghaee, S.; Doostan, M. Achillea wilhelmsii-incorporated chitosan@Eudragit nanoparticles intended for enhanced ulcerative colitis treatment. AAPS PharmSciTech 2023, 24, 112. https://doi.org/10.1208/s12249-023-02568-x.

43. Yipel, M.; Tekeli, İ.O.; Altinok-Yipel, F.; Coşkun, P.; Aslan, A.; Güvenç, M.; Beyazit, N.; Özsoy, Ş.Y. The protective effect of boswellic acid- and ellagic acid-loaded, colon-targeted, pH-sensitive N-succinyl chitosan in an ulcerative colitis rat model. J. Drug Deliv. Sci. Technol. 2022, 68, 103023. https://doi.org/10.1016/j.jddst.2021.103023.

44. Hales, D.; Muntean, D.M.; Neag, M.A.; Kiss, B.; Ștefan, M.G.; Tefas, L.R.; Tomuță, I.; Sesărman, A.; Rațiu, I.A.; Porfire, A. Curcumin-loaded microspheres are effective in preventing oxidative stress and intestinal inflammatory abnormalities in experimental ulcerative colitis in rats. Molecules 2022, 27, 5680.

https://doi.org/10.3390/molecules27175680.

45. Jori, C.; Ahmad, A.; Kumar, A.; Kumar, B.; Ali, A.; Ali, N.; Tabassum, H.; Khan, R. Bioactive chitosan-BSA Maillard-derived chrysin-loaded nanoparticles: A gastroprotective, biomucoadhesive approach for enhanced oral therapy in ulcerative colitis. Carbohydr. Polym. 2025, 359, 123537.

https://doi.org/10.1016/j.carbpol.2025.123537

46. Fan, S.; Zhao, Y.; Yao, Y.; Shen, X.; Chai, X.; Li, J.; Pi, J.; Huang, X.; Jin, H.; Zhou, Z. Oral colon-targeted pH-responsive polymeric nanoparticles loading naringin for enhanced ulcerative colitis therapy. J. Transl. Med. 2024, 22, 878.

https://doi.org/10.1186/s12967-024-05662-1.

47. Abbas, S.M.; Sudheer, P. Development and evaluation of curcumin-Eudragit microsponges for anti-inflammatory therapy of the colon. Bull. Pharm. Sci. Assiut. Univ. 2024, 47, 789-803. https://doi.org/10.21608/bfsa.2024.308209.2219.

48. Liu, X.; Diao, N.; Song, S.; Wang, W.; Cao, M.; Yang, W.; Guo, C.; Chen, D. Inflammatory macrophage reprogramming strategy of fucoidan microneedles-mediated ROS-responsive polymers for rheumatoid arthritis. Int. J. Biol. Macromol. 2024, 271, 132442. https://doi.org/10.1016/j.ijbiomac.2024.132442.

49. Yuan, L.; Yao, L.; Ren, X.; Chen, X.; Li, X.; Xu, Y.; Jin, T. Cartilage defect repair in a rat model via a nanocomposite hydrogel loaded with melatonin-loaded gelatin nanofibers and menstrual blood stem cells: An in vitro and in vivo study. J. Mater. Sci. Mater. Med. 2024, 35, 55. https://doi.org/10.1007/s10856-024-06820-z.

50. Qian, D.; Xu, J.; Zhang, X.; Hu, F.; Cao, S.; Dong, Y.; Liu, X.; Yao, Y.; Yu, H.; Lu, Y.; Ma X.; Cheng K.; Zhao X.; Nie G.; Zhang X. Microenvironment self-adaptive nanomedicine promotes spinal cord repair by suppressing inflammation cascade and neural apoptosis. Adv. Mater. 2024, 36, e2307624.

https://doi.org/10.1002/adma.202307624.

51. Su, X.Z.; Qiao, E.Q.; Teng, G.J.; Xiong, F. Quaternary ammonium salt microspheres loaded with vascular disrupting agents for targeted interventional therapy of hepatocellular carcinoma. Acta Biomater. 2025, 203, 591-603.

https://doi.org/10.1016/j.actbio.2025.07.055.

52. Abdelfattah, A.; Aboutaleb, A.E.; Abdel-Aal, A.B.M.; Abdellatif, A.A.H.; Tawfeek, H.M.; Abdel-Rahman, S.I. Design and optimization of PEGylated silver nanoparticles for efficient delivery of doxorubicin to cancer cells. J. Drug Deliv. Sci. Technol. 2022, 71, 103347. https://doi.org/10.1016/j.jddst.2022.103347.

53. Yang, Z.; Zhao, Y.; Zhang, X.; Huang, L.; Wang, K.; Sun, J.; Chen, N.; Yin, W.; Chen, S.; Zhi, H.; Xue L.; An L.; Li R.; Dong H.; Xu J.; Li Y.; Li Y. Nano-mechanical immunoengineering: Nanoparticle elasticity reprograms tumor-associated macrophages via Piezo1. ACS Nano 2024, 18, 21221-21235. https://doi.org/10.1021/acsnano.4c04614.

54. Cheng, X.; Yu, P.; Zhou, X.; Zhu, J.; Han, Y.; Zhang, C.; Kong, L. Enhanced tumor homing of pathogen-mimicking liposomes driven by R848 stimulation: A new platform for synergistic oncology therapy. Acta Pharm. Sin. B 2022, 12, 924-938. https://doi.org/10.1016/j.apsb.2021.08.018.

55. Chen, Y.W.; He, A.C.; Huang, T.Y.; Lai, D.H.; Wang, Y.P.; Liu, W.W.; Kuo, W.T.; Hou, H.H.; Cheng, S.J.; Lee, C.Y.; Chuang WC; Chang CC.; Lee BS. Iontophoresis-enhanced buccal delivery of cisplatin-encapsulated chitosan nanoparticles for treating oral cancer in a mouse model. Int. J. Nanomed. 2024, 19, 10435-10453.

https://doi.org/10.2147/IJN.S475742.

56. Dinya, M.; Horváth, D. A magisztrális készítmények helyzete hazánkban. Gyógyszerészet 2004, 48, 147-151.

57. Da, J.; Li, Y.; Zhang, K.; Ren, J.; Wang, J.; Liu, X.; Liu, X.; Zhang, J.; Liu, L.; Zhang, W.; Zhang S.; Guo Y.; Zhang B.; Jin H. Functionalized Prussian blue nanozyme as dual-responsive drug therapeutic nanoplatform against maxillofacial infection via macrophage polarization. Int. J. Nanomed. 2022, 17, 5851-5868.

https://doi.org/10.2147/IJN.S385899.

58. Shi, R.; Li, H.; Jin, X.; Huang, X.; Ou, Z.; Zhang, X.; Luo, G.; Deng, J. Promoting re-epithelialization in an oxidative diabetic wound microenvironment using self-assembly of a ROS-responsive polymer and P311 peptide micelles. Acta Biomater. 2022, 152, 425-439. https://doi.org/10.1016/j.actbio.2022.09.017.

59. Asad, S.; Wegler, C.; Ahl, D.; Bergström, C.A.S.; Phillipson, M.; Artursson, P.; Teleki, A. Proteomics-informed identification of luminal targets for in situ diagnosis of inflammatory bowel disease. J. Pharm. Sci. 2021, 110, 239-250. https://doi.org/10.1016/j.xphs.2020.11.001.

60. Ahmed, W.; Li, S.; Liang, M.; Kang, Y.; Liu, X.; Gao, C. Multifunctional drug- and AuNRs-loaded ROS-responsive selenium-containing polyurethane nanofibers for smart wound healing. ACS Biomater. Sci. Eng. 2024, 10, 3946-3957. https://doi.org/10.1021/acsbiomaterials.4c00363.

61. van Straten, D.; Bimbo, J.F.; Hennink, W.E.; Vermonden, T.; Schiffelers, R.M. Nanoparticle-in-hydrogel delivery system for the sequential release of two drugs. Pharmaceutics 2025, 17, 127. https://doi.org/10.3390/pharmaceutics17010127.

62. Jia, Y.; Han, Y.; Zhang, Y.; Li, L.; Zhang, B.; Yan, X. Multifunctional type III recombinant human collagen-incorporated sodium alginate hydrogel with sustained release of extracellular vesicles for wound healing multimodal therapy in diabetic mice. Regen. Ther. 2024, 27, 329-341. https://doi.org/10.1016/j.reth.2024.03.010.

63. Teora, S.P.; Panavaité, E.; Sun, M.; Kiffen, B.; Wilson, D.A. Anisotropic, Hydrogel Microparticles as pH-Responsive Drug Carriers for Oral Administration of 5-FU. Pharmaceutics 2023, 15, 1380. https://doi.org/10.3390/pharmaceutics15051380.

64. Li, M.; Wang, Y.; Han, X.; Liu, Y.; Ma, M.; Zhang, L. Multifunctional polydopamine-based nanoparticles for dual-mode imaging-guided targeted therapy of lupus nephritis. Pharmaceutics 2022, 14, 1988. https://doi.org/10.3390/pharmaceutics14101988.

65. Fu, L.; Huo, S.; Lin, P.; Wang, J.; Zhao, J.; You, Y.; Nie, X.; Ding, S. Precise antibiotic delivery to the lung infection microenvironment boosts the treatment of pneumonia with decreased gut dysbiosis. Acta Biomater. 2024, 184, 352-367. https://doi.org/10.1016/j.actbio.2024.06.026.

66. Wang, S.; Zhai, Y.; Liu, M.; Cheng, Z.; Zhang, J.; Zhang, H.; Wang, H.; Hu, Y.; Li, C.; Luo, Z. A hydrogel-based drug delivery system reduces inflammation and oxidative stress to alleviate intervertebral disc degeneration. Acta Biomater. 2025, 203, 229-244. https://doi.org/10.1016/j.actbio.2025.07.033.

67. Zeng, Q.; Gong, Y.; Jiao, W.; Xu, J.; Chen, X.; Xu, R.; Liu, Y.; Liang, X.; Li, G.; Liu, J.; He L.; Li W.; Zhang X.; Chen X.; Chen W. Smart ultra-long-lasting sequentially triggerable and artfully implantable nozzle system for on-demand drug delivery for chronotherapy. Sci. Adv. 2025, 11, eadv8734. https://doi.org/10.1126/sciadv.adv8734.

68. Hu, C.; Qiu, Y.; Guo, J.; Cao, Y.; Li, D.; Du, Y. An oxygen supply strategy for sonodynamic therapy in tuberculous granuloma lesions using a catalase-loaded nanoplatform. Int. J. Nanomed. 2023, 18, 6257-6274.

https://doi.org/10.2147/IJN.S430019.

69. Liu, Y.; He, J.; Li, M.; Ren, K.; Zhao, Z. Inflammation-driven nanohitchhiker enhances postoperative immunotherapy by alleviating prostaglandin E2-mediated immunosuppression. ACS Appl. Mater. Interfaces 2024, 16, 6879-6893. https://doi.org/10.1021/acsami.3c17357.

70. Tan, X.; Zhou, Y.; Qin, Y.; Wu, L.; Yang, R.; Bao, X.; Jiang, R.; Sun, X.; Ying, X.; Ben, Z.; Dai Q.; Zhang Z.; Zeng K.; Han M. Self-healing hydrogel resulting from the noncovalent interaction between ropivacaine and low-molecular-weight gelator sodium deoxycholate achieves stable and endurable local analgesia in vivo. ACS Appl. Mater. Interfaces 2024, 16, 45969-45988. https://doi.org/10.1021/acsami.4c07883.

71. Hu, D.; Huang, Z.; Li, W.; Shan, L.; Wu, M.Y.; Feng, S.; Wan, Y. Macrophage membrane-cloaked ROS-responsive albumin nanoplatforms for targeted delivery of curcumin to alleviate acute liver injury. Mol. Pharm. 2025, 22, 771-786.

https://doi.org/10.1021/acs.molpharmaceut.4c00808.

72. Qiu, X.; Yu, Y.; Liu, H.; Li, X.; Sun, W.; Wu, W.; Liu, C.; Miao, L. Remodeling the periodontitis microenvironment for osteogenesis by using a reactive oxygen species-cleavable nanoplatform. Acta Biomater. 2021, 135, 593-605.

https://doi.org/10.1016/j.actbio.2021.08.009.

73. Deng, Y.; Deng, X.; Li, Y.; Tian, J.; Wu, M.; Tang, J.; Liang, X.; Yang, X.; He, X.; Liu, Y.; Liu Z.; Zhou X.; Li T.; Li C. Size-adjustable nanoparticles co-target macrophages and endothelial cells for enhanced atherosclerosis therapy. Colloids Surf. B Biointerfaces 2025, 255, 114952. https://doi.org/10.1016/j.colsurfb.2025.114952.

74. Zhang, W.; Liu, Y.; Wang, Z.; He, S.; Liu, W.; Wu, Y.; Yang, L.; Hu, C.; Wang, Y. Remodeling brain pathological microenvironment to lessen cerebral ischemia injury by multifunctional injectable hydrogels. J. Control. Release 2024, 369, 591-603. https://doi.org/10.1016/j.jconrel.2024.03.050.

75. Chen, X.; Wu, Y.; Song, P.; Feng, L.; Zhou, Y.; Shi, J.; Dong, N.; Qiao, W. Matrix metalloproteinase-responsive controlled release of self-assembly nanoparticles accelerates heart valve regeneration in situ by orchestrating immunomodulation. Adv. Sci. 2025, 12, e2403351. https://doi.org/10.1002/advs.202403351.

76. Jia, Y.; Kong, X.; Li, R.; Wang, H.; Li, C.; Cheng, S.; Duan, W.; Xiao, Y.; Mai, Y.; Deng, W.; Liu Y. Enhanced nasal-to-brain drug delivery by multivalent bioadhesive nanoparticle clusters for cerebral ischemic reperfusion injury protection. Acta Biomater. 2025, 194, 411-427. https://doi.org/10.1016/j.actbio.2025.01.036.

77. Guo, W.; Hu, C.; Wang, Y.; Zhang, W.; Zhang, S.; Peng, J.; Wang, Y.; Wu, J. NO-releasing double-crosslinked responsive hydrogels accelerate the treatment and repair of ischemic stroke. Acta Pharm. Sin. B 2025, 15, 1112-1125.

https://doi.org/10.1016/j.apsb.2025.01.005.

78. Qin, X.; Zhu, L.; Zhong, Y.; Wang, Y.; Wu, G.; Qiu, J.; Wang, G.; Qu, K.; Zhang, K.; Wu, W. Spontaneously right-side-out-orientated coupling-driven ROS-sensitive nanoparticles on cell membrane inner leaflet for efficient renovation in vascular endothelial injury. Adv. Sci. 2023, 10, e2205093.

https://doi.org/10.1002/advs.202205093.

79. Wei, B.; Chen, L.; Huang, X.; Chi, F.; Li, G.; Yang, L.; Zheng, C.; Wang, Y. Microenvironment-responsive biomimetic bioprosthetic valve with antithrombosis and immunoregulation performance. ACS Appl. Mater. Interfaces 2025, 17, 18160-18178. https://doi.org/10.1021/acsami.5c01314.

80. Shen, M.; Yao, S.; Li, S.; Wu, X.; Liu, S.; Yang, Q.; Du, J.; Wang, J.; Zheng, X.; Li, Y. A ROS- and shear stress dual-sensitive bionic system with cross-linked dendrimers for atherosclerosis therapy. Nanoscale 2021, 13, 20013-20027.

https://doi.org/10.1039/d1nr05355h.

81. Wang, C.; Xiao, Z.; Fan, J.; Zhang, C.; Wang, T.; Qiu, Z.; Ye, F.; Chen, M.; Li, Y. Nanocarriers loaded with danshensu for treating ischemic stroke by reducing oxidative stress and glial overactivation. ACS Omega 2024, 9, 35686-35694. https://doi.org/10.1021/acsomega.4c03991.

82. Mudigonda, J.; Onohara, D.; Amedi, A.; Suresh, K.S.; Kono, T.; Corporan, D.; Padala, M. In vivo efficacy of a polymer-layered decellularized matrix composite as a cell-homing cardiovascular tissue substitute. Mater. Today Bio 2022, 17, 100451. https://doi.org/10.1016/j.mtbio.2022.100451.

83. Jin, L.; Zhu, Z.; Hong, L.; Qian, Z.; Wang, F.; Mao, Z. ROS-responsive 18β-glycyrrhetic acid-conjugated polymeric nanoparticles mediate neuroprotection in ischemic stroke through HMGB1 inhibition and microglia polarization regulation. Bioact. Mater. 2022, 19, 38-49. https://doi.org/10.1016/j.bioactmat.2022.03.040.

84. Zhang, Y.; Zhang, H.; Zhao, F.; Jiang, Z.; Cui, Y.; Ou, M.; Mei, L.; Wang, Q. Mitochondrial-targeted and ROS-responsive nanocarrier via nose-to-brain pathway for ischemic stroke treatment. Acta Pharm. Sin. B 2023, 13, 5107-5120.

9. Bibliography of the candidate's publications

I. Original publications related to the topic of the dissertation

Dinya, Mariann ✉; Dinya, Elek; Mórotz, Gábor M. ✉ From Pathology to Formulation: Designing Biodegradable Polymers for Personalized Drug Delivery. **Pharmaceutics** 18 (3), Paper 330, 23 p. (2026). DOI, WoS, Scopus, PubMed. IF: 6.9

Békési, G. ✉; Heinle, H.; Kakucs, R.; Pázmány, T.; Szombath, D.; **Dinya, M**.; Tulassay, Z.; Fehér, J.; Rácz, K.; Székács, B.; Riss, E.; Farkas, A.; Gódor, F.; Illyés, G. Effect of inhibitors of myeloperoxidase on the development of aortic atherosclerosis in an animal model. **Experimental Gerontology** 40 (3), 199-208. (2005). IF: 3.008

Dinya, M. ✉; Székely, E.; Szentmihályi, K.; Tasnádi, Gy.; Blázovics, A. Major and trace elements in whole blood of phlebotomized patients with porphyria cutanea tarda. **Journal of Trace Elements in Medicine and Biology** 19 (2-3), 217-220. (2005). IF: 0.955

Dinya, Mariann; Antal, István Intelligens polimerek alkalmazása a gyógyszer-technológiában. 1. rész. Általános fogalmak, lehetőségek. **Gyógyszerészet** 46 (2), 67-71. (2002).

Antal, István; **Dinya, Mariann** Intelligens polimerek alkalmazása a gyógyszer-technológiában. 2. rész. A hatóanyagleadás szabályozására alkalmas mechanizmusok. **Gyógyszerészet** 46 (3), 141-145. (2002).

II. Original publications not directly related to the topic of the dissertation

Bradács, Melinda; **Dinya, Mariann**; Stampf, György. Egy szakdolgozat tanulságai: gyógyszerészi pályatükör a hálózati kollégák és a hallgatók szemével. **Gyógyszerészet** 51 (8), 473-479. (2007).

Dinya, Mariann; Horváth, Dénes. Konfekció vagy egyedi darab? A magisztrális készítmények helyzete hazánkban. **Gyógyszerészet** 48 (3), 147-151. (2004).

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pharmaceutics

MDPI

Systematic Review

From Pathology to Formulation: Designing Biodegradable Polymers for Personalized Drug Delivery

Mariann Dinya [1,2,*], Elek Dinya [3] and Gábor M. Mórotz [1,2,*]

[1] Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, H-1089 Budapest, Hungary
[2] Center for Pharmacology and Drug Research & Development, Semmelweis University, H-1089 Budapest, Hungary
[3] Institute of Digital Health Sciences, Semmelweis University, H-1089 Budapest, Hungary; dinya.elek@semmelweis.hu
* Correspondence: dinya.mariann.katalin@semmelweis.hu (M.D.); morotz.gabor@semmelweis.hu (G.M.M.)

Highlights

What are the main findings?

- Analysis of 65 in vivo studies reveal disease-specific polymer–trigger patterns
- Pathological microenvironments guide rational polymer carrier design.

What are the implications of the main findings?

- Ionizable polysaccharide and methacrylate systems dominate intestinal inflammation.
- Enzyme- and redox-responsive polymers align with joint and tumor diseases.
- Multi-responsive carriers improve robustness in heterogeneous environments.

Abstract

Background/Objectives: Selection of polymer carriers for targeted drug delivery is typically guided by material availability or trigger responsiveness rather than disease-specific evidence. However, successful preclinical formulations may already encode implicit design rules linking polymer composition to particular pathological environments. This study aimed to identify reproducible material-disease associations across biodegradable polymer systems and to derive formulation-oriented guidance for disease-calibrated carrier selection. **Methods:** A structured synthesis of 65 preclinical in vivo studies (2020–2025) covering inflammatory bowel disease, arthritis, cardiovascular inflammation, and solid tumors was performed. Extracted variables included polymer family, backbone chemistry, stimulus responsiveness, disease model, and reported therapeutic benefit relative to controls. Associations between polymer composition, trigger mechanisms, and disease categories were analyzed using cross-tabulation, chi-square statistics, Cramér's V, and direction-of-effect synthesis. **Results:** Distinct material-disease clustering patterns emerged. Ionizable polysaccharide and methacrylate systems (e.g., alginate, chitosan, Eudragit) were strongly associated with intestinal inflammatory models, reflecting reliance on pH- and ion-mediated mechanisms. Enzyme-degradable hyaluronic acid matrices were concentrated in joint and cartilage disorders characterized by protease overexpression. Oxidation-sensitive polyether systems (e.g., PEG-PPS) and redox-active hybrid platforms predominated in atherosclerosis and tumor models, where oxidative stress is a defining pathological feature. Composite and multi-responsive systems were disproportionately represented in tumors, consistent with microenvironmental heterogeneity. Across studies, therapeutic improvement was consistently reported when polymer functional motifs aligned with dominant biochemical drivers of the disease. **Conclusions:** Successful biodegradable polymer

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carriers exhibit disease-specific compatibility patterns rather than universal applicability. These recurring associations suggest that polymer selection can be guided by pathological context even in the absence of direct outcome comparisons. The resulting formulation-oriented framework supports rational carrier choice for personalized drug delivery based on disease-specific microenvironment signatures.

Keywords: biodegradable polymers; pathological microenvironment; disease-driven design

1. Introduction

Modern pharmaceutical manufacturing, particularly in generic drug development, relies on a relatively small repertoire of well-characterized polymer excipients selected primarily for safety, scalability, regulatory acceptance, and manufacturing feasibility. Although these materials enable robust and reproducible formulations, they are frequently applied across diverse therapeutic areas without explicit consideration of disease-specific microenvironmental conditions [1–13]. Consequently, material selection for targeted therapies often remains largely empirical, despite evidence that pathological tissues exhibit markedly altered biochemical environments, including changes in pH, redox balance, enzymatic activity, and ionic composition. Targeted drug-delivery systems aim to enhance therapeutic efficacy while minimizing systemic toxicity by concentrating pharmacological activity at diseased sites. Biodegradable polymer carriers have become central to this strategy because they allow controlled drug release through diffusion, matrix degradation, and stimulus-responsive mechanisms. In particular, stimuli-responsive systems exploit endogenous pathological cues such as extracellular acidity, oxidative stress, protease activity, or inflammation-associated biochemical changes to achieve site-specific activation. pH-responsive methacrylate polymers and polysaccharide matrices have enabled localized intestinal drug delivery, while redox-responsive platforms target oxidative stress across inflammatory, ischemic, and neoplastic tissues [14–19]. Enzyme-responsive matrices further extend this concept by coupling drug release to disease-associated protease activity. Matrix metalloproteinase-responsive hydrogels, for example, have been developed for both osteoarthritis and inflammatory bowel disease, where elevated protease levels correlate with tissue destruction and inflammation [20,21]. Such systems demonstrate that pathological microenvironments can serve as functional triggers for drug delivery rather than passive barriers.

Different diseases present distinct microenvironmental signatures, motivating the development of tailored carrier systems. Inflammatory bowel disease has driven extensive research into pH-sensitive, microbiota-responsive, and mucoadhesive platforms capable of distal intestinal targeting [3–7,12]. Cardiovascular inflammation and atherosclerosis have been addressed using reactive oxygen species (ROS)-responsive nanoplatforms, including shear-sensitive designs that respond to oxidative stress and altered hemodynamics within plaques [18,22,23]. Protease-rich joint environments have been targeted using enzyme-degradable hydrogels and hyaluronic-acid-based matrices designed for sustained intra-articular release [20,21,24]. In oncology, multifunctional carriers combining pH, redox, or metal-ion sensitivity seek to address tumor heterogeneity and dynamic microenvironmental conditions [25–27]. Despite rapid progress, most formulation strategies are developed within individual disease models and interpreted in isolation. As a result, knowledge embedded in successful systems, such as recurring material choices for specific pathological environments, remains fragmented across the literature. Polymer classes exhibit characteristic physicochemical behaviors that may confer advantages in particular microenvironments,

yet whether these theoretical compatibilities translate into reproducible patterns of material selection across studies has not been systematically examined.

Direct comparison of therapeutic efficacy across preclinical models is complicated by heterogeneity in animal species, disease induction methods, outcome measures, and dosing regimens. Nevertheless, the consistent observation of therapeutic benefit relative to controls suggests that the literature collectively represents a repository of experimentally validated design solutions. Analyzing this landscape may reveal implicit formulation principles guiding the development of disease-targeted carriers.

The present study synthesizes evidence from 65 preclinical in vivo investigations published between 2020 and 2025 across inflammatory bowel disease, arthritis and cartilage disorders, cardiovascular inflammation, and solid tumors. Rather than comparing efficacy across heterogeneous models, we examined associations between polymer composition, stimulus-responsive mechanisms, and disease context to identify recurring material-disease pairings. Our objectives were to determine which classes of biodegradable polymers are most frequently associated with specific pathological conditions, to assess whether single- or multi-responsive systems predominate in different environments, and to derive formulation-oriented guidance that may support rational carrier selection for context-dependent and potentially personalized therapeutic strategies.

2. Materials and Methods

2.1. Search Strategy, Data Sources, and Eligibility Criteria

A systematic literature search was conducted in the PubMed and Scopus databases to identify preclinical in vivo studies published between 1 January 2020 and 31 August 2025.

PubMed and Scopus were selected because together they provide comprehensive coverage of biomedical, pharmacological, and materials science literature relevant to stimulus-responsive polymer drug-delivery systems, with substantial overlap with other major indexing databases.

The search strategy combined controlled vocabulary and free-text terms addressing three main domains: biodegradable polymeric drug delivery systems, stimulus responsiveness (including ion-, pH-, ROS-, enzyme-, or dual-responsive mechanisms), and animal models of inflammatory or neoplastic diseases. The full Boolean search strings for each database are provided below to ensure reproducibility.

The search was performed independently by two reviewers (M.D. and G.M.M.) using the same predefined strategy in duplicate rounds across both databases to maximize retrieval completeness and minimize selection bias. Inter-reviewer agreement during screening was assessed using Cohen's kappa coefficient, and disagreements were resolved by discussion or consultation with a third reviewer (E.D.).

The database search yielded 104 records (PubMed n = 62; Scopus n = 42). After removal of four duplicate entries, 100 records remained for title and abstract screening, of which 35 were excluded as clearly irrelevant. Sixty-five full-text articles met the predefined eligibility criteria and were included in the final analysis (Figure 1). This systematic review followed the PRISMA 2020 guidelines [28].

PUBMED search query:

("polymer matrix"[tiab] OR "polymeric matrix"[tiab] OR hydrogel[tiab] OR microsphere*[tiab] OR nanoparticle*[tiab])

AND ("drug delivery"[tiab] OR "controlled release"[tiab] OR "sustained release"[tiab] OR "modified release"[tiab] OR "drug release"[tiab])

AND ((blood[tiab] OR serum[tiab] OR plasma[tiab])

AND (“ion environment”[tiab] OR “ionic environment”[tiab] OR “ionic microenvironment”[tiab] OR “electrolyte”[tiab] OR “ionic strength”[tiab] OR sodium[tiab] OR potassium[tiab] OR calcium[tiab] OR magnesium[tiab] OR phosphate[tiab])

AND (inflammation[tiab] OR colitis[tiab] OR arthritis[tiab] OR atherosclerosis[tiab] OR sepsis[tiab] OR hepatitis[tiab] OR pancreatitis[tiab])

AND (in vivo[tiab] OR animal*[tiab] OR mouse[tiab] OR rat[tiab] OR rabbit[tiab] OR murine[tiab]) NOT (review[pt] OR “systematic review”[tiab] OR “meta-analysis”[tiab] OR case reports[pt] OR ocular[tiab] OR ophthalmic[tiab] OR wound[tiab] OR transdermal[tiab]) AND (“1 January 2020”[Date—Publication] : “31 August 2025”[Date—Publication])

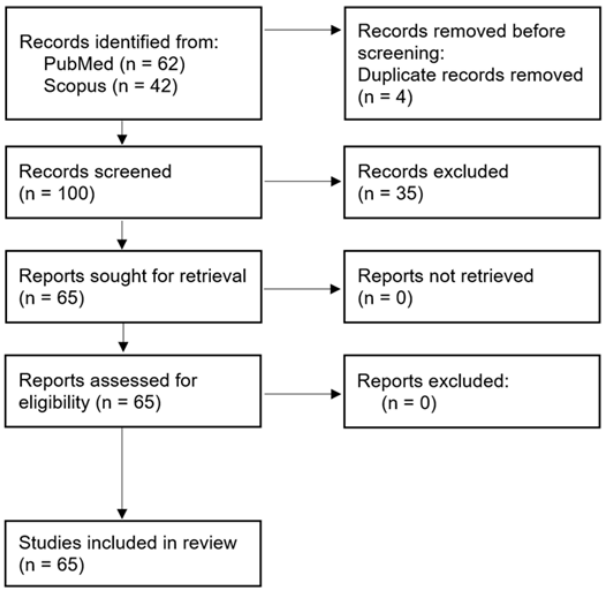

Figure 1. PRISMA flow diagram of study selection. Database searches were conducted in PubMed and Scopus databases. Identification yielded 104 records (PubMed: 8 + 54; Scopus: 27 + 15). After removing 4 duplicates, 100 records were screened at the title/abstract level, and 35 were excluded. In total, 65 studies were included in the qualitative synthesis. Reasons for exclusion are summarized in the Methods section.

SCOPUS search query:

TITLE(“polymer matrix” OR “polymeric matrix” OR hydrogel OR microsphere OR nanoparticle) AND TITLE-ABS-KEY(“drug delivery” OR “controlled release” OR “sustained release” OR “modified release” OR “drug release”) AND TITLE-ABS-KEY((blood OR serum OR plasma) AND (human OR patient* OR clinical OR volunteer* OR cohort OR mouse OR mice OR murine OR rat OR rats OR rabbit*)) AND TITLE-ABS-KEY((“ionic strength” OR electrolyte* OR sodium OR potassium OR calcium OR magnesium OR phosphate OR “ion environment” OR “ionic environment” OR “ionic microenvironment” OR “electrolyte environment” OR “ionic milieu” OR “electrolyte milieu”) W/5 (release OR “drug release” OR “controlled release”)) AND TITLE-ABS-KEY(inflammation OR inflammatory OR colitis OR arthritis OR “inflammatory bowel disease” OR atherosclerosis OR sepsis OR hepatitis OR pancreatitis OR cancer OR tumor OR carcinoma OR neoplasm*)AND PUBYEAR > 2019 AND PUBYEAR < 2026AND LIMIT-TO(DOCTYPE, “ar”)AND LIMIT-TO(LANGUAGE, “English”)

Eligibility criteria were defined according to the Population-Intervention-Comparator-Outcome (PICO) framework (Table 1). Studies were included if they investigated biodegradable polymer-based carriers with stimulus-responsive drug release in preclinical in vivo models of inflammatory or neoplastic disease, regardless of delivery route, and were reported as original research articles. Studies were excluded if they were reviews, meta-

analyses, or conference abstracts; used non-biodegradable or non-polymeric carriers; focused exclusively on wound healing, ocular, or transdermal applications; or reported only in vitro data without in vivo validation. Articles not meeting the inclusion criteria for the primary analysis could nevertheless be cited elsewhere for contextual purposes.

Table 1. PICO framework applied in the present systematic review.

PICO Element	Description
Population (P)	Preclinical in vivo animal models and supplementary ex vivo/human serum studies in cancer and inflammatory conditions (e.g., colitis, hepatitis, atherosclerosis, sepsis, tumor models)
Intervention (I)	Ion- or pH-sensitive biodegradable polymer carriers (e.g., Eudragit, alginate, PLA/PLGA, pH-sensitive hydrogels, ROS-responsive polymers)
Comparator (C)	Non-sensitive polymer carriers/free drug/placebo control
Outcome (O)	Therapeutic efficacy (tumor size, inflammatory markers, survival), and the relationship between efficacy and host electrolyte milieu

The search strategy was intentionally designed to capture studies investigating microenvironment-responsive polymer systems across inflammatory and neoplastic conditions, including those influenced by ionic, pH, redox, or enzymatic factors, without restricting the search to specific polymer classes or delivery platforms.

2.2. Data Extraction

Data were independently extracted from all eligible studies by the same two reviewers described above using a standardized spreadsheet developed specifically for this analysis. Extracted variables included bibliographic information (first author, publication year, journal), disease model characteristics (species, induction method, pathological subtype), polymer type and formulation features, primary stimulus responsiveness (pH, ion, ROS/redox, enzyme, dual/multi-responsive, or other), route of administration, therapeutic endpoints (e.g., tumor volume, histological score, inflammatory markers, oxidative stress indicators, survival), and quantitative outcome measures where available (means, standard deviations, standard errors, *p*-values, or confidence intervals).

Discrepancies were resolved by discussion and, when necessary, adjudication within the author team. All numerical data were extracted directly from the text or tables of the original publications; graphical digitization was not performed. Because of substantial heterogeneity across disease models, polymer chemistries, stimulus mechanisms, and reported endpoints, quantitative synthesis was feasible only for subsets of studies with sufficient comparability.

To capture broader structure-function relationships across heterogeneous experimental designs, a complementary descriptive framework was applied. This included vote counting and direction-of-effect assessment to summarize outcome patterns across polymer families, trigger types, and disease categories. Given that most included studies represented proof-of-concept investigations reporting therapeutic improvement relative to controls, outcomes were classified primarily according to the presence or absence of reported benefit. For mapping analyses, formulations demonstrating biologically or statistically meaningful improvement over control conditions were categorized as positive outcomes, whereas neutral or negative findings were recorded only when explicitly reported. This approach enabled consistent evaluation of associations between material design parameters and therapeutic performance rather than direct comparison of efficacy across fundamentally different models.

Classification of Stimulus Responsiveness

Stimulus responsiveness was classified according to the primary release-driving mechanism explicitly described in each full-text article. Each formulation was assigned to one predefined category based on the dominant mechanism governing in vivo drug activation: pH-responsive, ROS/redox-responsive, enzyme-responsive, ion-responsive, dual or multi-responsive, or other mechanisms.

pH-responsive systems included formulations activated by protonation-deprotonation processes, pH-dependent solubility transitions, acid-labile linkages, or enteric dissolution behavior. ROS-responsive systems comprised carriers destabilized by oxidative stress through redox-labile bonds or oxidation-induced structural changes. Enzyme-responsive systems required enzymatic cleavage or degradation mediated by disease-associated enzymes such as matrix metalloproteinases, hyaluronidase, or microbial enzymes. Ion-responsive systems were classified as such only when ion exchange or ion-dependent crosslink dissociation was explicitly identified as the primary trigger of drug release.

Formulations exhibiting multiple responsiveness (e.g., pH+ROS, pH+ion, ROS+enzyme) were categorized as dual or multi-responsive when at least two stimuli contributed to activation. Constituent stimulus components were additionally recorded to enable secondary mapping analyses of trigger combinations. Intrinsic ionic effects inherent to polymer structure (e.g., polyelectrolyte swelling or charge screening) were not considered independent triggers unless explicitly identified by the original authors as the principal release mechanism. Systems driven primarily by targeting ligands, biomimetic coatings, physical cues, or other non-canonical mechanisms were assigned to the "other" category.

Classification decisions were based on the descriptions provided in the original studies and were applied consistently across all included formulations.

Detailed characteristics of the included studies and extracted parameters are presented in Supplementary Table S1. All included studies are listed and referenced in Supplementary Table S1 [14–27,29–79].

2.3. Risk of Bias Assessment

Risk of bias in individual studies was evaluated using a modified version of the SYRCLE tool for animal experiments [3]. The assessment covered key methodological domains including randomization procedures, allocation concealment, blinding of investigators and outcome assessors, sample size reporting, completeness of outcome data, and selective reporting.

Given the exploratory nature and substantial heterogeneity of the included preclinical studies, the bias assessment was used primarily to identify methodological limitations and potential sources of uncertainty rather than to exclude studies from the analysis.

2.4. Statistical Analysis

Where quantitative outcome data were sufficiently comparable, effect sizes were calculated as standardized mean differences (Hedges' g) with 95% confidence intervals using a random-effects model (DerSimonian–Laird method).

Between-study heterogeneity was assessed using Cochran's Q statistic, degrees of freedom (df), the H statistic ($H = \sqrt{(Q/df)}$), I^2 ($I^2 = \max\{0,(Q - df)/Q\} \times 100\%$), and τ^2. Approximate thresholds of 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively [4].

2.4.1. Effect Sizes, Subgroups, and Sensitivity

Data extraction included polymer type, stimulus-responsiveness category, disease model, route of administration, and primary therapeutic outcomes as reported by the original authors.

Pre-specified subgroup analyses examined formulation type (nanoparticle, hydrogel, microsphere), stimulus-responsiveness category, disease group (inflammatory vs. tumor), and route of administration (oral vs. parenteral). Sensitivity analyses included leave-one-out procedures and Baujat influence plots. Publication bias and small-study effects were evaluated using contour-enhanced funnel plots and Egger's regression. Where asymmetry was suggested ($p < 0.10$), trim-and-fill analyses were performed.

2.4.2. Sparse Data and Association Analyses

This hybrid analytical approach was adopted to accommodate the heterogeneity of preclinical models and the frequent absence of extractable quantitative parameters required for conventional meta-analysis.

Because many studies did not report extractable quantitative parameters, additional analyses were performed to identify structural relationships within the dataset. Vote counting and direction-of-effect plots were used to summarize outcome trends across studies. Associations between polymer families, trigger mechanisms, and disease categories were analyzed using chi-square tests, Cramér's V, and standardized residuals.

2.4.3. Overall Associations and Analytical Considerations

Across the 65 included studies, global associations between trigger type, polymer family, and disease category were evaluated using chi-square statistics and Cramér's V. Standardized residual analyses were performed to identify localized over-representations within contingency tables. Vote counting was used to estimate the proportion of studies reporting positive, neutral, or negative outcomes across disease groups and trigger categories. Because most studies were proof-of-concept investigations reporting therapeutic improvement relative to controls, this approach enabled consistent comparison of outcome patterns across heterogeneous models.

Although a random-effects meta-analysis was prespecified, calculation of pooled effect sizes was not feasible for the majority of studies due to insufficient numerical reporting, with many outcomes presented only graphically. Therefore, association analyses and descriptive synthesis were prioritized, while the meta-analytic framework was retained for potential future updates if extractable data become available.

3. Results

3.1. Study Selection and Characteristics

To analyze the most recent advances in microenvironment-responsive drug delivery, our systematic search across PubMed and Scopus focused on studies published between 2020 and 2025. The query identified 104 records. After the removal of four duplicates, 100 articles were screened by title and abstract. Full texts of potentially eligible articles were retrieved and assessed for compliance with the inclusion criteria. Thirty-five studies were excluded due to ocular delivery, wound-healing indications, non-biodegradable materials, or absence of in vivo validation. Ultimately, 65 studies met the inclusion criteria and were included in the final synthesis (Figure 1).

The selected studies covered a wide spectrum of inflammatory and tumor-related disease models, including IBD, arthritis and cartilage disorders, atherosclerosis, hepatitis, pancreatitis, and solid tumors. Both murine and larger animal models (mouse, rat, rabbit) were employed, using standard induction methods such as DSS-induced colitis, collagen-

induced arthritis, high-fat diet-induced atherosclerosis, and xenograft implantation. Across these models, formulations were based on biodegradable polymer systems with diverse backbone chemistries, including methacrylate copolymers (e.g., Eudragit), polysaccharides (chitosan, alginate, and hyaluronic acid), aliphatic polyesters (PLGA), gelatin-based matrices, PEG-derived block copolymers, and hybrid biomimetic constructs.

Many platforms combined natural and synthetic components, membrane coatings, or inorganic modules to achieve disease-specific responsiveness. Stimulus sensitivity encompassed pH, ion, ROS, and enzyme triggers, as well as dual- or multi-responsive systems, reflecting attempts to match carrier behavior to pathological microenvironments. Administration routes were predominantly oral and parenteral (intravenous, intraperitoneal, intratumoral).

In cardiovascular diseases, particularly in atherosclerosis, multiple studies focused on ROS-responsive platforms. ROS-responsive nanoassemblies enhanced macrophage targeting and attenuated atherosclerotic plaque progression [18,19,27,37,62], while cell-membrane-based and shear-stress-responsive systems promoted endothelial repair and plaque stabilization in ApoE$^{-/-}$ models [22,60,62]. In acute myocardial infarction, inflammation-modulating nanoparticle systems reduced infarct size and myocardial fibrosis by suppressing inflammatory pathways and macrophage activation [53,79]. Microenvironment-responsive hydrogels have also been engineered for cardiovascular implants and tissue substitutes, where glycocalyx-mimetic or polymer-layered coatings improved antithrombosis, immunoregulation, and resistance to calcification [61,70].

In joint and cartilage disorders, including rheumatoid arthritis, osteoarthritis, and intervertebral disk degeneration disease, polymeric hydrogels responsive to matrix metalloproteinases (MMPs) and hyaluronic-acid-based composites were frequently applied. These systems enabled enzyme-triggered degradation and targeted intra-articular drug release, supporting localized therapy with reduced systemic exposure across multiple in vivo arthritis and cartilage repair models [7,20,24,34,49,54,55,58].

Tumor models, such as hepatocellular carcinoma, colorectal cancer, and breast cancer, highlighted the versatility of dual-responsive carriers. In colorectal cancer, ROS- and enzyme-sensitive nanoparticles improved drug penetration and tumor inhibition [35]. For hepatocellular carcinoma, polymer-based targeted nanocarriers provided selective release in the tumor microenvironment while reducing systemic toxicity [36,71].

Some excluded studies still provided contextual insights, such as applications in wound healing, ocular delivery, or non-biodegradable polymer systems [8–11].

3.2. Quantitative Synthesis and Mapping

The analyses in Section 3.2 describe patterns of application across studies (frequencies and associations) rather than comparative therapeutic efficacy.

3.2.1. Trigger Type Distribution

First, we quantified the distribution of stimulus-responsive mechanisms across the 65 included studies using the harmonized trigger classification described in Section 2.2. ROS-responsive systems were reported in 23 studies (35.4%), pH-responsive platforms in 20 studies (30.8%), and dual- or multi-responsive formulations in 12 studies (18.5%). Approaches categorized as “other”, including ligand-mediated or mechanically responsive strategies, were reported in 6 studies (9.2%), while enzyme-responsive carriers appeared in 4 studies (6.2%) (Figure 2).

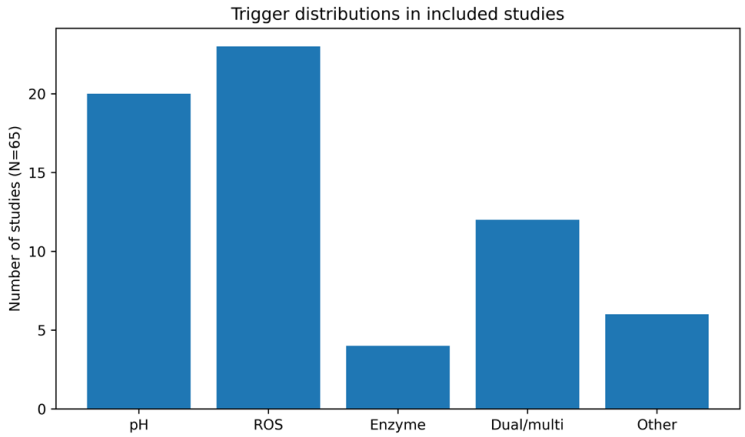

Figure 2. Trigger distribution across the 65 analyzed studies. ROS, reactive oxygen species.

3.2.2. Polymer Family Trigger Type Mapping

Cross-tabulation of polymer families and stimulus types (Figure 3) revealed non-uniform responsiveness patterns across the included studies. Eudragit-based systems were pH-responsive (8 studies, 12.3%). Chitosan- and alginate-based carriers appeared primarily in pH-responsive categories and were also represented in ROS-responsive and multi-trigger formulations. PEG-PPS copolymers clustered within ROS-activated systems (4 studies, 6.2%). Hyaluronic acid (HA) platforms were represented mainly in ROS-related and dual-responsive designs (3 studies, 4.6%). PLGA-based carriers showed distribution across pH-, ROS-, and multi-responsive strategies (6 studies, 9.2%). MOF- and nanozyme-containing systems were linked mainly to ROS-dependent or combined triggers (3 studies, 4.6%).

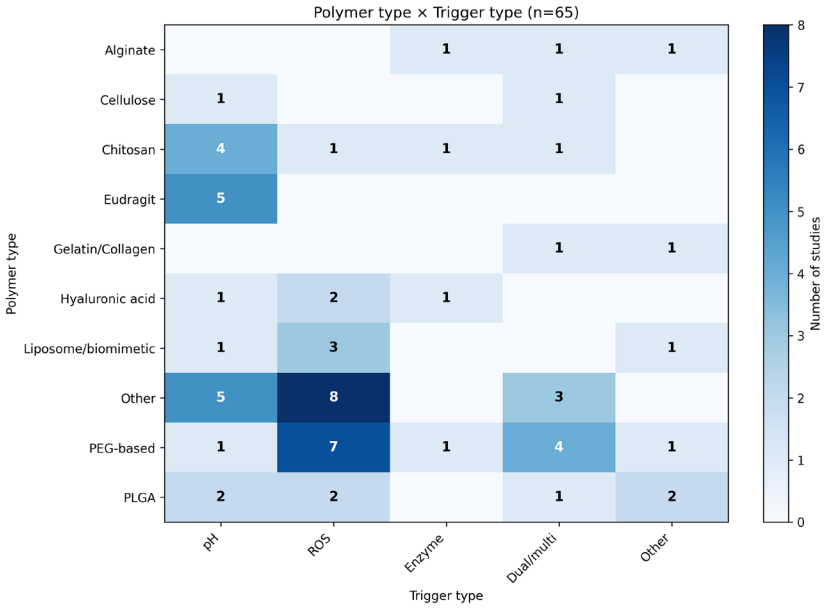

Figure 3. Polymer type-trigger type mapping across the 65 analyzed studies based on harmonized classifications. The heatmap shows the frequency of polymer classes (rows) associated with different stimuli-responsiveness (columns). Darker shading indicates higher study counts. PEG-based, poly(ethylene glycol)-derived copolymers; PLGA, poly(lactic-co-glycolic acid); ROS, reactive oxygen species.

Taken together, Figure 3 shows that polymer families were not evenly distributed across trigger categories, with some materials appearing predominantly in single-trigger designs and others recurring across multiple trigger types.

3.2.3. Cross-Tabulation Analysis of Polymer Chemistry and Trigger Type

To further resolve the patterns observed at the polymer family level, stimulus distribution was analyzed according to polymer backbone chemistry (Figure 4). Composite or biomimetic hybrid systems were represented across ROS-responsive and multi-trigger designs. Polyanionic methacrylate copolymers (e.g., Eudragit) appeared predominantly in pH-responsive studies, while polycationic polysaccharides (mainly chitosan-based systems) were likewise dominated by pH-driven release. Anionic polysaccharides were more frequently represented in multi-responsive designs. Aliphatic polyesters (PLGA/PLA) were distributed across ROS-responsive and combined-trigger categories. Polyethers, including PEG-derived copolymers such as PEG-PPS, clustered within ROS-activated systems.

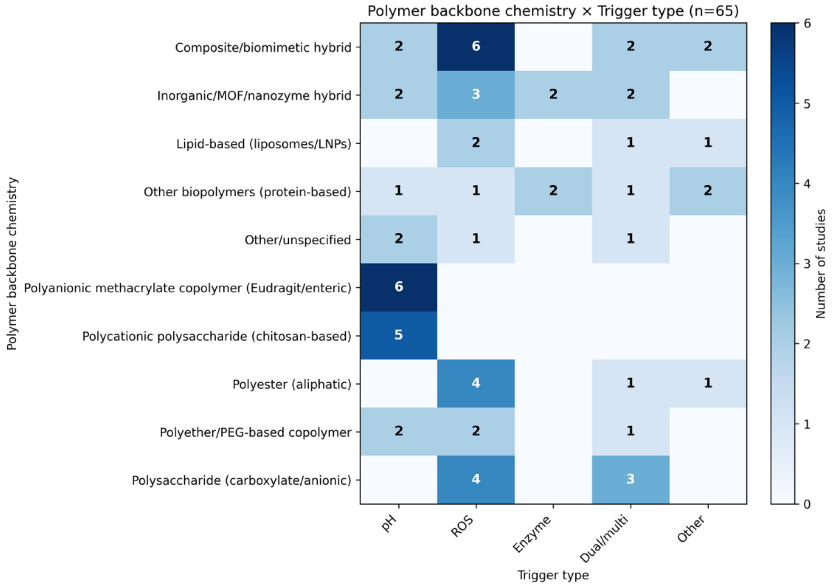

Figure 4. Cross-tabulation of polymer backbone chemistry and trigger type across the 65 included studies. The heatmap shows the frequency of associations between polymer chemistry classes (rows) and stimulus-responsive mechanisms (columns). Numbers indicate the number of studies for each combination, and darker shading corresponds to higher study counts. Dual/multi categories represent formulations responsive to more than one stimulus (e.g., pH+ROS, pH+ion, ROS+enzyme). MOF, metal–organic framework; ROS, reactive oxygen species.

Inorganic or catalytic materials, including MOF- and nanozyme-containing systems, were concentrated in ROS-related and combined categories. Crosslinked polymer networks appeared mainly in pH- and enzyme-responsive systems, consistent with cleavage- or degradation-controlled release. Protein-based matrices were rare and showed no dominant trigger profile.

Overall, Figure 4 shows that stimulus responsiveness follows polymer backbone chemistry rather than carrier form. Polyelectrolytes align with pH-dependent mechanisms, oxidation-sensitive polyethers with ROS triggers, degradable polyesters support broader strategies, and hybrid systems enable multi-trigger designs.

3.2.4. Trigger Distribution Across Disease Groups

We analyzed the distribution of trigger types across disease groups to identify disease-specific design patterns (Figure 5). Inflammatory models, including colitis, arthritis, atherosclerosis, hepatitis, and pancreatitis, were predominantly associated with pH-responsive systems. ROS-responsive platforms were also frequently applied in inflammatory diseases, particularly in atherosclerosis models. Ion-responsive systems and enzyme-

sensitive carriers were less frequent but were primarily confined to inflammatory contexts, especially gastrointestinal and joint disorders.

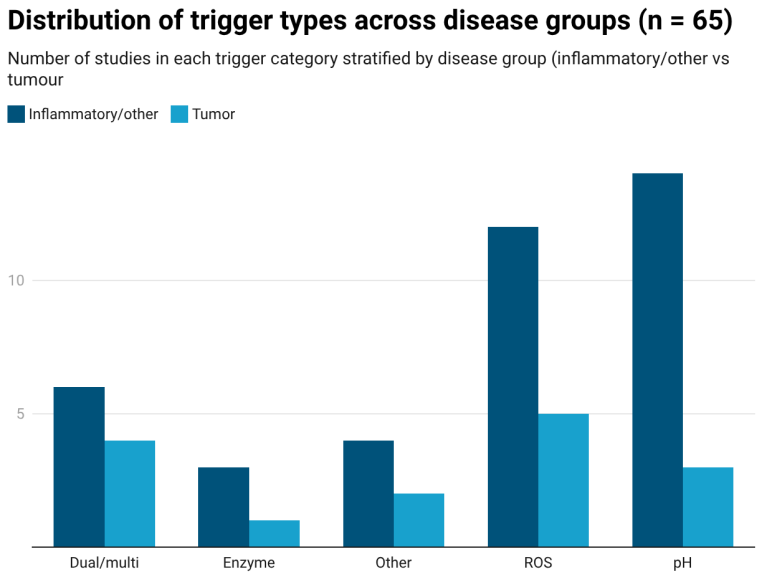

Figure 5. Distribution of trigger types across disease groups in the 65 included studies. Bars represent the number of studies employing each trigger mechanism in inflammatory versus tumor models. ROS, reactive oxygen species.

In contrast, tumor models were dominated by ROS-responsive and dual-responsive designs. Enzyme-responsive systems appeared only sporadically in tumor settings, whereas ion-associated mechanisms were rare outside inflammatory conditions.

Overall, trigger profiles differed between inflammatory and tumor models, with pH-associated mechanisms recurring more frequently in inflammatory conditions and ROS-based or multi-responsive systems recurring more frequently in tumor-targeted formulations (Figure 5).

3.2.5. Polymer Chemistry Distribution Across Disease Groups

Polymer backbone chemistry showed a clear disease-dependent distribution across the included studies (Figure 6). Composite and biomimetic hybrid systems constituted the largest category overall and were strongly enriched in inflammatory models. Polyanionic methacrylate copolymers (e.g., Eudragit) and protein-based matrices were likewise almost exclusively associated with inflammatory diseases.

In contrast, inorganic and MOF/nanozyme hybrid systems displayed a comparatively higher representation in tumor models, distinguishing them from most organic polymer classes. Aliphatic polyesters, polyethers, polysaccharide-based carriers, and lipid systems were present in both disease groups.

Other or unspecified systems were rare and showed no consistent disease preference. Overall, these data indicate that polymer selection is not uniform across indications but shows distinct distributions between inflammatory versus tumor-targeted applications (Figure 6).

To clarify how stimulus selection relates to specific pathological environments, trigger distributions were analyzed across disease subtypes (Figure 7). Distinct patterns emerged across disease subtypes. Across inflammatory conditions, pH- and ROS-responsive systems predominated, but their relative contributions differed substantially between subtypes.

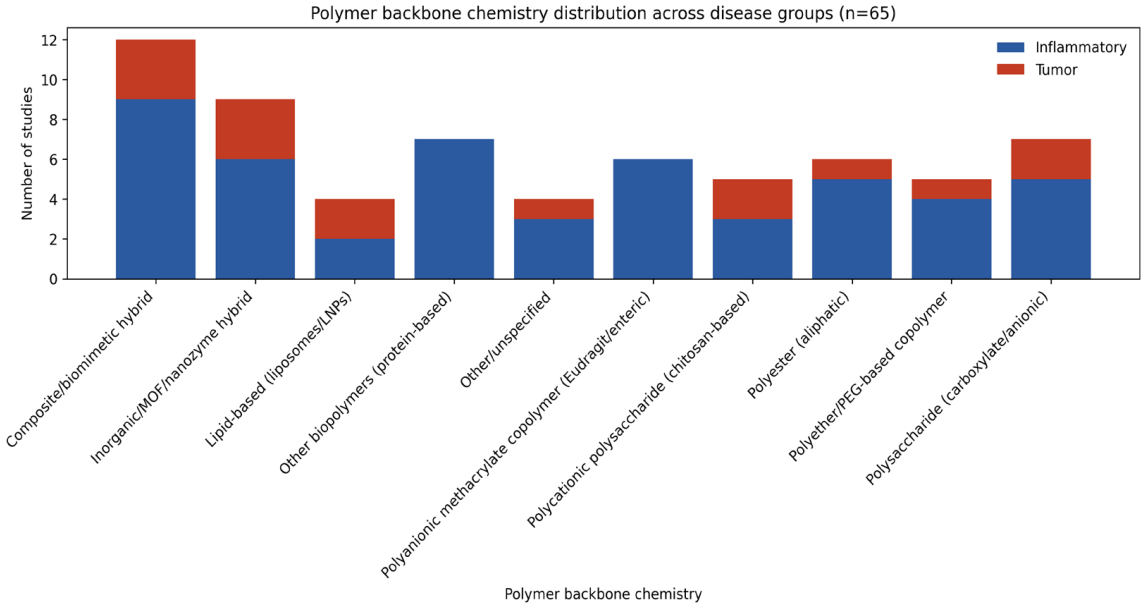

Figure 6. Polymer chemistry distribution across disease groups across 65 included studies. The stacked bar chart illustrates the number of studies focusing on inflammatory diseases (blue) or solid tumors (red) in relation with polymer chemistry. MOF, metal–organic frameworks.

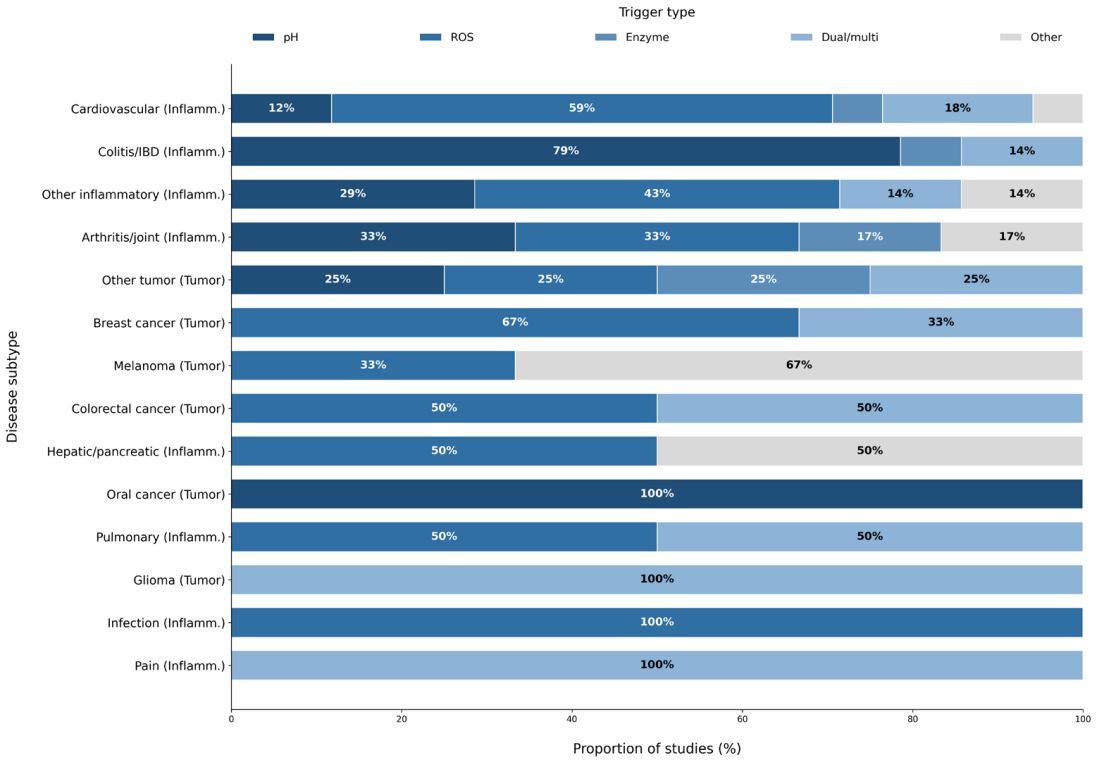

Figure 7. Normalized trigger-type distribution across inflammatory and tumor disease subtypes (n = 65). Stacked bars represent the percentage contribution of pH-, ROS-, enzyme-, dual/multi-, and other stimulus-responsive systems within each subtype. Normalization enables comparison of trigger selection patterns across disease contexts with unequal sample sizes.

Cardiovascular inflammatory models were dominated by ROS-responsive systems. These studies [19,27,37,41,42,60,62,64,75,79] primarily employed redox-responsive polymers or hybrid carrier systems.

Colitis and inflammatory bowel disease models showed a strong bias toward pH-responsive platforms. Protonation-dependent behavior of ionizable polymers enabled

localized drug release within the inflamed colon. Arthritis and joint disorders displayed a broader trigger profile. pH-responsive systems were represented by studies [24,34], ROS-responsive systems by [7,61], enzyme-responsive platforms by [20], and alternative trigger strategies by [58], indicating heterogeneous trigger usage across joint disease models. Other inflammatory conditions exhibited mixed trigger usage across studies.

Tumor models showed fewer studies overall but demonstrated clear subtype-specific tendencies. Breast cancer models (n = 3) were restricted to oxidative and dual-responsive designs, with ROS-responsive systems represented by [25,32] and dual/multi-responsive platforms by [23]. Melanoma models were associated primarily with non-classical or composite triggers; for example, study [52] utilized a pathogen-mimicking platform categorized as "Other".

Colorectal cancer models relied on both ROS-responsive and dual-responsive carriers, represented by studies [26,35], respectively. Oral cancer models included pH-responsive systems such as [38].

Across inflammatory and tumor subtypes, trigger categories showed subtype-dependent distributions, with pH-, ROS-, enzyme-, and dual/multi-responsive systems recurring at different frequencies (Figure 7).

3.2.6. Polymer Type-Trigger-Disease Interaction Network

Finally, we integrated the relationships between polymer families, dominant stimulus-responsiveness categories, and associated disease models into a tripartite network (Figure 8). The resulting network revealed distinct clusters among the analyzed groups.

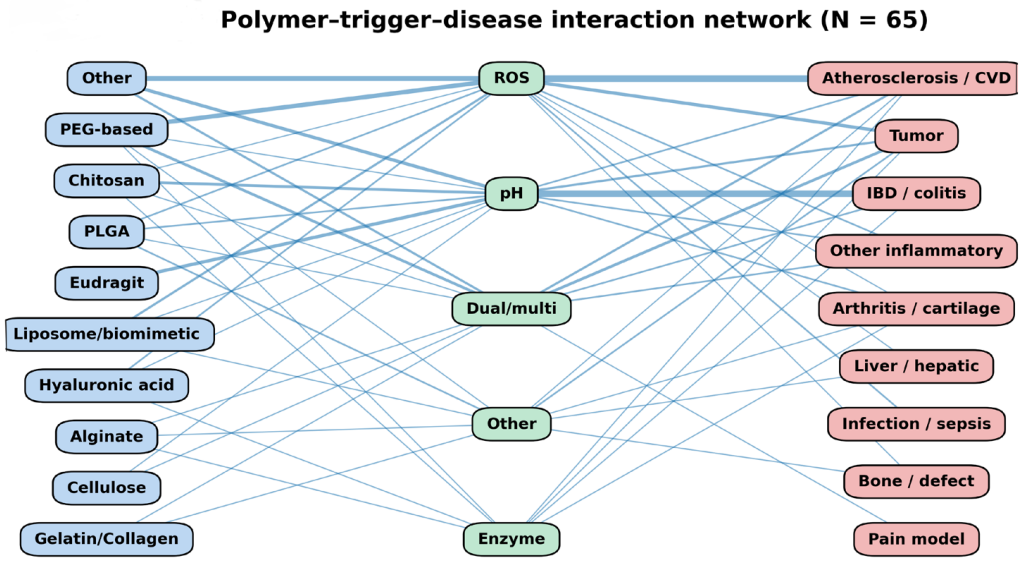

Figure 8. Polymer-trigger-disease interaction network. The tripartite network illustrates the relationships between biodegradable polymer families (blue nodes), dominant trigger mechanisms (green nodes), and associated disease model groups (red nodes) across 65 included preclinical studies (n = 65). Edge thickness corresponds to the number of studies supporting each association. The map highlights dominant formulation strategies linking polymer chemistry, microenvironmental responsiveness, and disease context.

Polyanionic methacrylate copolymers (Eudragit), together with chitosan- and alginate-based systems, were predominantly associated with pH- and ion-responsive triggers and were mainly linked to inflammatory bowel disease models. PEG-PPS and MOF/nanozyme-based systems formed a separate cluster around ROS-responsive triggers and were strongly associated with tumor and atherosclerosis models. HA-based hydrogels were mapped

primarily to enzyme-responsive triggers and were most frequently applied in arthritis and intervertebral disk degeneration models.

Connections between multiple triggers and disease groups were observed for dual- or multi-responsive systems, indicating cross-category associations across heterogeneous pathological conditions.

Overall, the network representation demonstrates non-random associations between polymer families, trigger mechanisms, and disease models across the included studies.

4. Discussion

4.1. Integration of Outcomes

Rather than testing whether microenvironment-responsive polymers can function, the present analysis addresses a more practice-oriented question: Which material strategies have been repeatedly applied with reported benefit across diverse pathological settings? Because the majority of included studies reported improvement relative to untreated or baseline controls, a pattern that may partly reflect publication tendencies toward positive findings in preclinical research, the dataset represents a "solution landscape" of experimentally validated formulations rather than a balanced comparison between success and failure. This perspective enables identification of recurring design patterns linking polymer chemistry, trigger mechanism, and disease microenvironment.

Across disease models, therapeutic alignment appears structured rather than random. Outcome patterns stratified by polymer family correspond to characteristic functional groups and degradation pathways: ionizable carboxylates and amines in methacrylates, alginate, and chitosan support protonation–deprotonation equilibria and ionic swelling; ester hydrolysis governs PLGA behavior in vascular and tumor contexts; redox-active thioethers in PEG-PPS and labile coordination bonds in metal–organic frameworks enable ROS-sensitive destabilization; and glycosidic hyaluronic acid backbones are susceptible to enzyme-mediated cleavage in protease-rich tissues. These recurring motifs suggest physicochemical compatibility between polymer backbones and disease-associated microenvironmental features.

Importantly, trigger categories did not perform uniformly across contexts. Protonation equilibria and ion-exchange mechanisms showed the most consistent representation in colitis models, whereas ROS-responsive platforms predominated in tumor and atherosclerosis settings. Enzyme-responsive systems were concentrated in joint and degenerative models. Dual- or multi-responsive designs appeared across heterogeneous conditions and were frequently associated with robust therapeutic readouts, suggesting that combined activation mechanisms may buffer spatial and temporal variability within diseased tissues.

Viewed through a network framework, polymer–trigger–disease relationships form coherent clusters rather than diffuse associations. Polysaccharide- and methacrylate-based carriers converge on pH- and ion-sensitive strategies in inflammatory bowel disease; PEG–PPS systems and metal–organic frameworks cluster within oxidative tumor and vascular environments; hyaluronic-acid hydrogels map predominantly to enzyme-responsive joint disorders. Multi-node connections highlight the recurring use of composite materials in complex microenvironments.

Based on these patterns, Table 2 consolidates empirically recurrent polymer–trigger combinations by disease subtype together with their mechanistic rationale. Rather than prescribing a universally optimal material, this synthesis delineates a structured design space within which polymer selection can be aligned with dominant pathological conditions.

Table 2. Recommended polymer-trigger systems by disease subtype, associated positive outcome rates, and mechanistic rationale.

Disease Subtype	Recommended Polymer Family/System	Dominant Trigger Mechanism	Evidence Strength *	Mechanistic Rationale
IBD/colitis	Eudragit (methacrylate copolymers)	pH-responsive	Strong	Enteric dissolution and protonation-deprotonation equilibria enable site-specific release in acidic inflamed intestinal regions
	Chitosan-based systems	pH/ionic	Moderate	Cationic swelling, mucoadhesion, and electrostatic interaction with negatively charged mucosa enhance retention and release
	PLGA formulations	pH-associated degradation	Supportive	Ester hydrolysis and autocatalytic acidification promote sustained release under inflammatory conditions
Arthritis/cartilage disorders	Hyaluronic acid hydrogels	Enzyme- responsive (MMPs, hyaluronidase)	Strong	Selective degradation in protease-rich synovial environment enables localized drug liberation
	Alginate or HA composites	ROS/enzyme	Moderate	Oxidative stress and enzymatic activity jointly promote matrix breakdown in inflamed joints
Atherosclerosis/cardiovascular disease	ROS-responsive polymers (e.g., PEG-PPS)	ROS	Strong	High oxidative burden in plaques activates redox- sensitive linkages, triggering payload release
	PEGylated nanoparticle systems	ROS/dual	Moderate	Prolonged circulation combined with oxidative activation enhances vascular targeting
Solid tumours	ROS-responsive systems	ROS	Strong	Elevated ROS flux in tumour microenvironment ensures efficient activation of redox- labile carriers
	Dual pH + ROS platforms	Combined stimuli	Moderate-strong	Concurrent acidity and oxidative stress provide robust activation despite spatial heterogeneity

* Evidence strength reflects the frequency of successful application across included studies rather than comparative therapeutic efficacy. "Positive outcome" denotes any statistically or biologically meaningful improvement in disease-related endpoints reported by the original authors relative to control conditions in the respective preclinical model. Across the included studies, authors reported improvement relative to control conditions; negative or null findings may be underrepresented due to publication and reporting biases.

Taken together, the findings support the concept of mechanochemical compatibility as a central organizing principle in biodegradable polymer drug delivery. By integrating evidence across models, this work provides a formulation-oriented framework that moves beyond descriptive cataloging toward context-aware material selection.

4.2. Translational Relevance of Recommended Polymer-Trigger Systems

Table 2 synthesizes the most consistently reported polymer-trigger pairings across 65 preclinical studies, providing a formulation-oriented framework for disease-specific carrier selection. Rather than identifying universally superior materials, the data indicate that therapeutic performance is strongly influenced by the degree of mechanochemical compatibility between the carrier and the dominant pathological microenvironment.

In colitis and inflammatory bowel disease, polyanionic methacrylate copolymers (Eudragit) and polysaccharide matrices such as alginate and chitosan were frequently associated with reproducible outcomes. These systems exploit robust physicochemical constants of the intestinal milieu: protonation–deprotonation equilibria along the colonic pH gradient and dynamic ion-exchange processes. Carboxylate-bearing methacrylates undergo predictable dissolution transitions, while Ca^{2+}-crosslinked alginate networks respond to Na^{+}/Ca^{2+} exchange through formation and rearrangement of egg-box junction zones. Combined with chitosan-mediated mucoadhesion and ionic swelling, these

mechanisms provide stable, scalable colon-targeted delivery platforms compatible with oral administration.

In arthritis and degenerative joint disorders, hyaluronic-acid-based hydrogels and HA-PEG composites were consistently associated with positive performance by leveraging matrix metalloproteinase (MMP) overexpression in inflamed synovial tissues. PEGylation improves retention and reduces nonspecific protein adsorption, whereas enzymatic cleavage of the HA backbone enables localized payload release. This trigger mechanism closely mirrors disease pathophysiology and supports the development of intra-articular depot formulations capable of sustained therapeutic exposure.

Atherosclerosis and vascular inflammation were dominated by ROS-responsive carriers, including PEG-PPS micelles and MOF- or nanozyme-based composites. Oxidation of thioether groups and destabilization of labile coordination bonds under oxidative stress can induce structural collapse and drug release. However, variability in local ROS concentrations suggests that adaptive or multi-responsive systems may be required for reliable human translation.

Solid tumors frequently employ dual pH+ROS-responsive or biomimetic composite systems. Their orthogonal activation pathways enable drug release even when one stimulus is heterogeneous or subthreshold, while nanoscale size and surface properties facilitate accumulation via the enhanced permeability and retention (EPR) effect. This redundancy may be particularly advantageous in spatially heterogeneous tumor microenvironments.

Collectively, these findings emphasize that polymeric drug-delivery systems tend to perform most consistently when chemical functionality, material physics, and disease-specific triggers are co-optimized. Translation is therefore unlikely to benefit from universal carrier platforms; instead, rational selection should prioritize alignment between ionizable groups, redox-sensitive motifs, or enzyme-labile structures and the dominant biochemical cues of the target pathology. Proteomics-derived epithelial biomarkers (e.g., TGM2, ICAM1, CEACAM1, ANXA1 [9]) further support the integration of disease-specific diagnostic profiling with targeted or trigger-matched formulation strategies in IBD.

4.3. Mechanistic Interpretation

Across stimuli-responsive platforms, efficacy patterns appear to closely track the intrinsic chemical reactivity of the materials within physiological ranges of pH, ionic strength, and redox potential. Polyanionic methacrylates exhibit predictable solubility shifts driven by protonation of pendant carboxyl groups, whereas polycationic polysaccharides such as chitosan rely on reversible amine protonation to induce swelling and mucoadhesion. Polyester carriers including PLGA and PLA undergo hydrolysis that is accelerated under acidic and inflammatory conditions, explaining enhanced release kinetics in tumors and damaged tissues.

Redox-responsive block copolymers such as PEG-PPS convert thioether oxidation into polarity changes that destabilize micellar cores, directly coupling drug release to oxidative stress gradients characteristic of atherosclerotic plaques and tumors. Hyaluronic-acid matrices exploit enzyme overexpression for site-specific degradation, while MOF- and nanozyme-based systems combine labile coordination bonds with catalytic modulation of reactive oxygen species, producing both therapeutic and environment-responsive effects.

From a translational perspective, these observations support the concept of mechano-chemical matching, defined as the alignment between molecular reactivity and pathological milieu as a major determinant of in vivo performance. Systems activated by fundamental physicochemical properties of disease states, such as acidity, oxidative stress, or protease activity, tend to exhibit more reproducible outcomes. In contrast, platforms dependent on less stable or spatially variable stimuli may produce inconsistent results across models.

Importantly, each polymer family offers a defined activation pathway that can be tuned through composition, architecture, or crosslinking density. This design flexibility may enable the prediction of behavior under patient-specific biochemical conditions and provide a foundation for personalized formulation strategies. At a molecular level, responsiveness is governed by a limited set of recurring chemical motifs, including ionizable carboxylate and amine groups enabling pH- and ion-dependent behavior; hydrolysable ester backbones driving acid-accelerated degradation; redox-active thioethers mediating oxidative destabilization; enzyme-cleavable glycosidic networks; and labile coordination bonds in metal–organic frameworks. These motifs collectively define the physicochemical design space of biodegradable polymer carriers.

4.4. Regulatory and Translational Challenges

Considerable heterogeneity among the included studies, encompassing animal models, polymer architectures, dosing strategies, and outcome metrics, limits direct quantitative comparability. Incomplete reporting of sample sizes, variance measures, and release kinetics further constrains formal effect-size synthesis and necessitates a hybrid qualitative–quantitative approach.

Translational extrapolation is additionally complicated by differences between animal models and human pathological microenvironments. Although rodent models reproduce major disease hallmarks, electrolyte composition, pH distribution, oxidative status, and protease activity often differ from human tissues, potentially altering trigger responsiveness.

Future progress will require integration of mechanistic polymer design with experimental models that better capture patient-level biochemical variability. Biomarker-guided selection of responsive motifs, including protonatable groups, ion-sensitive crosslinks, redox-labile bonds, and enzyme-cleavable backbones, represents a promising strategy to align carrier behavior with disease-specific environments.

In summary, this analysis maps a coherent landscape of disease-linked polymer responsiveness but also highlights the need for standardized reporting, improved quantitative characterization, and translational models that reflect clinically relevant biochemical conditions.

4.5. Limitations of the Study

Despite providing a comprehensive synthesis of microenvironment-responsive polymer systems, this analysis has several limitations. Considerable heterogeneity among the included studies, in terms of animal models, polymer architectures, dosing regimens, administration routes, and outcome measures, limits direct quantitative comparability across experiments. In addition, incomplete reporting of sample sizes, variance measures, pharmacokinetic parameters, and release kinetics in some studies constrained formal meta-analytic effect size calculations and necessitated a hybrid qualitative–quantitative approach.

Translational extrapolation from animal models to human disease remains uncertain. Although rodent models reproduce key pathological features, differences in electrolyte composition, pH distribution, oxidative status, immune responses, and protease activity may alter trigger responsiveness in clinical settings. Furthermore, publication bias toward positive findings in preclinical research may lead to overrepresentation of successful formulations.

Finally, the analysis focused exclusively on biodegradable polymer systems published between 2020 and 2025 and indexed in major databases, which may exclude earlier foundational work or studies reported in other sources. Therefore, the framework proposed here should be interpreted as a contemporary evidence-based landscape rather than an exhaustive representation of all possible responsive materials.

5. Conclusions

Analysis of 65 preclinical in vivo studies identified non-random, disease-specific clustering of biodegradable polymer systems according to dominant activation mechanisms. Rather than being broadly interchangeable, polymer platforms tended to converge within distinct pathological contexts, indicating that disease microenvironments impose strong constraints on viable material designs.

Gastrointestinal inflammatory models were overwhelmingly associated with ionizable polysaccharide-based and methacrylate carriers operating through pH- and ion-dependent mechanisms, whereas enzyme-responsive hyaluronic acid systems were concentrated in joint and cartilage pathologies characterized by protease overexpression. In contrast, oxidative vascular disorders and solid tumors preferentially employed redox-responsive polyether systems and coordination-chemistry-driven platforms, reflecting the central role of reactive oxygen species in these conditions. Notably, composite and multi-responsive carriers were disproportionately represented in disease settings known for spatial and temporal heterogeneity, suggesting that robustness across fluctuating microenvironments may require orthogonal activation pathways.

These findings indicate that therapeutic performance is associated not simply with trigger compatibility but with the stability and dominance of specific biochemical cues within each disease. Pathologies with relatively uniform activation signals tend to favor single-mechanism systems, whereas complex or evolving microenvironments are more frequently addressed by hybrid architectures capable of maintaining functionality despite local variability.

By consolidating these recurrent associations into a polymer–trigger–disease framework, this study provides a practical basis for disease-calibrated formulation selection. Polymer choice may therefore be guided by the dominant microenvironmental drivers of the target pathology rather than by empirical material preference. Such an approach has direct implications for translational development, as alignment between carrier responsiveness and disease biology is likely to influence reproducibility and clinical viability.

Collectively, these findings suggest that, unlike platform-based pharmaceutical formulation, effective experimental drug-delivery systems exhibit disease-dependent material selection patterns. Incorporating pathological context into carrier choice may therefore support more rational and potentially personalized formulation strategies.

This framework may serve as a preliminary decision-support tool for selecting polymer carriers in personalized formulation development.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmaceutics18030330/s1. Table S1. Characteristics of the included studies (n = 65), including study identifier, disease model, polymer type, stimulus type (pH/ROS/ion/enzyme/dual), final stimulus classification, in vivo model, main therapeutic endpoint, and proposed mechanism of responsiveness. Ref. [28] has cited in Supplementary Materials.

Author Contributions: M.D.: conceptualization, methodology, formal analysis, investigation, data curation, visualization, writing—original draft, writing—review and editing. E.D.: formal analysis, validation, writing—review and editing, biostatistical support. G.M.M.: supervision, validation, review and editing, founding acquisition. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

EPR	enhanced permeability and retention
HA	hyaluronic acid
IBD	inflammatory bowel disease
MMP	matrix metalloproteinase
MOF	metal–organic framework
PEG	poly(ethylene glycol)
PEG-PPS	poly(ethylene glycol)-block-poly(propylene sulfide)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
ROS	reactive oxygen species
Tg	glass transition temperature

References

1. Poole, K.M.; Nelson, C.E.; Joshi, R.V.; Martin, J.R.; Gupta, M.K.; Haws, S.C.; Kavanaugh, T.E.; Skala, M.C.; Duvall, C.L. ROS-responsive microspheres for on-demand antioxidant therapy in a model of diabetic peripheral arterial disease. *Biomaterials* **2015**, *41*, 166–175. [CrossRef] [PubMed]
2. Dinya, M.; Székely, E.; Szentmihályi, K.; Tasnádi, G.; Blázovics, A. Major and trace elements in whole blood of phlebotomized patients with porphyria cutanea tarda. *J. Trace Elem. Med. Biol.* **2005**, *19*, 217–220. [CrossRef] [PubMed]
3. Hooijmans, C.R.; Rovers, M.M.; de Vries, R.B.; Leenaars, M.; Ritskes-Hoitinga, M.; Langendam, M.W. SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* **2014**, *14*, 43. [CrossRef]
4. Higgins, J.P.; Thompson, S.G.; Deeks, J.J.; Altman, D.G. Measuring inconsistency in meta-analyses. *BMJ* **2003**, *327*, 557–560. [CrossRef]
5. McKenzie, J.E.; Brennan, S.E.; Ryan, R.E. Synthesizing and presenting findings using other methods. In *Cochrane Handbook for Systematic Reviews of Interventions*, Version 6.3; Higgins, J.P.T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M.J., Welch, V.A., Eds.; Cochrane: London, UK, 2022.
6. Egger, M.; Davey Smith, G.; Schneider, M.; Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **1997**, *315*, 629–634. [CrossRef]
7. Liu, X.; Diao, N.; Song, S.; Wang, W.; Cao, M.; Yang, W.; Guo, C.; Chen, D. Inflammatory macrophage reprogramming strategy of fucoidan microneedles-mediated ROS-responsive polymers for rheumatoid arthritis. *Int. J. Biol. Macromol.* **2024**, *271*, 132442. [CrossRef] [PubMed]
8. Shi, R.; Li, H.; Jin, X.; Huang, X.; Ou, Z.; Zhang, X.; Luo, G.; Deng, J. Promoting re-epithelialization in an oxidative diabetic wound microenvironment using self-assembly of a ROS-responsive polymer and P311 peptide micelles. *Acta Biomater.* **2022**, *152*, 425–439. [CrossRef] [PubMed]
9. Asad, S.; Wegler, C.; Ahl, D.; Bergström, C.A.S.; Phillipson, M.; Artursson, P.; Teleki, A. Proteomics-informed identification of luminal targets for in situ diagnosis of inflammatory bowel disease. *J. Pharm. Sci.* **2021**, *110*, 239–250. [CrossRef]
10. Abdelfattah, A.; Aboutaleb, A.E.; Abdel-Aal, A.B.M.; Abdellatif, A.A.H.; Tawfeek, H.M.; Abdel-Rahman, S.I. Design and optimization of PEGylated silver nanoparticles for efficient delivery of doxorubicin to cancer cells. *J. Drug Deliv. Sci. Technol.* **2022**, *71*, 103347. [CrossRef]
11. Ahmed, W.; Li, S.; Liang, M.; Kang, Y.; Liu, X.; Gao, C. Multifunctional drug- and AuNRs-loaded ROS-responsive selenium-containing polyurethane nanofibers for smart wound healing. *ACS Biomater. Sci. Eng.* **2024**, *10*, 3946–3957. [CrossRef]

12. van Straten, D.; Bimbo, J.F.; Hennink, W.E.; Vermonden, T.; Schiffelers, R.M. Nanoparticle-in-hydrogel delivery system for the sequential release of two drugs. *Pharmaceutics* **2025**, *17*, 127. [CrossRef]
13. Jia, Y.; Han, Y.; Zhang, Y.; Li, L.; Zhang, B.; Yan, X. Multifunctional type III recombinant human collagen-incorporated sodium alginate hydrogel with sustained release of extracellular vesicles for wound healing multimodal therapy in diabetic mice. *Regen. Ther.* **2024**, *27*, 329–341. [CrossRef]
14. Cai, X.; Wang, X.; He, M.; Wang, Y.; Lan, M.; Zhao, Y.; Gao, F. Colon-targeted delivery of tacrolimus using pH-responsive polymeric nanoparticles for murine colitis therapy. *Int. J. Pharm.* **2021**, *606*, 120836. [CrossRef]
15. Liu, H.; Cai, Z.; Wang, F.; Hong, L.; Deng, L.; Zhong, J.; Wang, Z.; Cui, W. Colon-Targeted Adhesive Hydrogel Microsphere for Regulation of Gut Immunity and Flora. *Adv. Sci.* **2021**, *8*, e2101619. [CrossRef]
16. Feng, J.; Wang, Y.; Lv, Y.; Fang, S.; Ren, M.; Yao, M.; Lan, M.; Zhao, Y.; Gao, F. XA pH-Responsive and Colitis-Targeted Nanoparticle Loaded with Shikonin for the Oral Treatment of Inflammatory Bowel Disease in Mice. *Mol. Pharm.* **2022**, *19*, 4157–4170. [CrossRef] [PubMed]
17. Ali, A.S.; Altayari, A.A.; Khan, L.M.; Alharthi, S.E.; Ahmed, O.A.; El-Shitany, N.A.; Ali, S.S.; Saadah, O.I. Colon-targeted therapy of tacrolimus (FK506) in the treatment of experimentally induced colitis. *Pharmacology* **2020**, *105*, 541–549. [CrossRef]
18. Shen, M.; Li, H.; Yao, S.; Wu, X.; Liu, S.; Yang, Q.; Zhang, Y.; Du, J.; Qi, S.; Li, Y. Shear stress- and ROS-responsive biomimetic micelles for atherosclerosis via ROS consumption. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2021**, *126*, 112164. [CrossRef] [PubMed]
19. He, J.; Zhang, W.; Zhou, X.; Xu, F.; Zou, J.; Zhang, Q.; Zhao, Y.; He, H.; Yang, H.; Liu, J. Reactive oxygen species (ROS)-responsive size-reducible nanoassemblies for deeper atherosclerotic plaque penetration and enhanced macrophage-targeted drug delivery. *Bioact. Mater.* **2022**, *19*, 115–126. [CrossRef]
20. Yi, X.; Xu, Z.; Liu, Q.; Zhou, H.; Yuan, L.; Li, D.; Zhao, L.; Mu, C.; Ge, L. Matrix metalloproteinase-responsive collagen-oxidized hyaluronic acid injectable hydrogels for osteoarthritic therapy. *Biomater. Adv.* **2022**, *137*, 212804. [CrossRef]
21. Xie, X.; Wang, Y.; Deng, B.; Blatchley, M.R.; Lan, D.; Xie, Y.; Lei, M.; Liu, N.; Xu, F.; Wei, Z. Matrix metalloproteinase-responsive hydrogels with tunable retention for on-demand therapy of inflammatory bowel disease. *Acta Biomater.* **2024**, *186*, 354–368. [CrossRef] [PubMed]
22. Shen, M.; Jiang, H.; Zhao, Y.; Wu, L.; Yang, H.; Yao, Y.; Meng, H.; Yang, Q.; Liu, L.; Li, Y. Shear stress- and ROS dual-responsive RBC-hitchhiking nanoparticles for atherosclerosis therapy. *ACS Appl. Mater. Interfaces* **2023**, *15*, 43374–43386. [CrossRef] [PubMed]
23. Da, J.; Li, Y.; Zhang, K.; Ren, J.; Wang, J.; Liu, X.; Liu, X.; Zhang, J.; Liu, L.; Zhang, W.; et al. Functionalized Prussian blue nanozyme as dual-responsive drug therapeutic nanoplatform against maxillofacial infection via macrophage polarization. *Int. J. Nanomed.* **2022**, *17*, 5851–5868. [CrossRef]
24. Li, H.; Lyu, Y.; Wang, R.; Yu, H.; Lin, M.; Li, Z.; Zhong, Y.; Sheng, P.; Zhang, K.; Liao, W.; et al. Supramolecular drug-laden hydrogel based on structural tautomerization enhances drug delivery for rheumatoid arthritis treatment. *Bioact. Mater.* **2025**, *53*, 495–506. [CrossRef] [PubMed]
25. Cheng, F.; Wang, S.; Zheng, H.; Yang, S.; Zhou, L.; Liu, K.; Zhang, Q.; Zhang, H. Cu-doped cerium oxide-based nanomedicine for tumor microenvironment-stimulative chemo-chemodynamic therapy with minimal side effects. *Colloids Surf. B Biointerfaces* **2021**, *205*, 111878. [CrossRef]
26. Zheng, S.; Guo, Y.; Han, Q.; Peng, X.; Sheng, R.; Liu, S.; Li, Z. STING agonists and PI3Kγ inhibitor co-loaded ferric ion-punicalagin networks for comprehensive cancer therapy. *Int. J. Biol. Macromol.* **2024**, *282*, 136776. [CrossRef]
27. Yang, H.; Guo, M.; Guan, Q.; Zhang, L.; Liu, M.; Li, H.; Qiao, G.; Yang, Q.; Shen, M.; Li, Y. ROS-responsive simvastatin nano-prodrug based on tertiary amine-oxide zwitterionic polymer for atherosclerotic therapy. *J. Nanobiotechnol.* **2025**, *23*, 176; Erratum in *J. Nanobiotechnol.* **2025**, *23*, 277. https://doi.org/10.1186/s12951-025-03359-1. [CrossRef]
28. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef] [PubMed]
29. Chen, R.; Xia, Y.; Ma, Z.; Ke, Y.; Shi, Q.; Ataullakhanov, F.I.; Panteleev, M. H_2O_2-responsive polymeric micelles of biodegradable aliphatic poly(carbonate)s as promising therapeutic agents for inflammatory diseases. *Macromol. Biosci.* **2024**, *24*, e2300409. [CrossRef]
30. Lin, Y.; Fang, S.; Chen, X.; Li, X.; Zhao, X.; Wang, Y.; Lu, J.; Ji, Q.; Zheng, S.; Zou, J.; et al. Pectinase-responsive nanoplatform for oral targeted delivery of 5-aminosalicylic acid and zinc coordination-driven nanozyme in inflammatory bowel disease treatment. *Int. J. Pharm. X* **2025**, *10*, 100362. [CrossRef]
31. Zeeshan, M.; Ain, Q.U.; Weigmann, B.; Story, D.; Smith, B.R.; Ali, H. Dual pH and microbial-sensitive galactosylated polymeric nanocargoes for multi-level targeting to combat ulcerative colitis. *Asian J. Pharm. Sci.* **2023**, *18*, 100831. [CrossRef] [PubMed]
32. Poonia, N.; Lather, V.; Narang, J.K.; Beg, S.; Pandita, D. Resveratrol-loaded folate-targeted lipoprotein-mimetic nanoparticles with improved cytotoxicity, antioxidant activity and pharmacokinetic profile. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2020**, *114*, 111016. [CrossRef]

33. Zhao, Y.; Xu, C.; Liu, Q.; Lei, X.; Deng, L.; Wang, F.; Yang, J. pH-responsive interface conversion efficient oral drug delivery platform for alleviating inflammatory bowel disease. *Front. Chem.* **2024**, *12*, 1365880. [CrossRef]
34. Phan, V.H.G.; Murugesan, M.; Nguyen, P.P.T.; Luu, C.H.; Le, N.H.; Nguyen, H.T.; Manivasagan, P.; Jang, E.S.; Li, Y.; Thambi, T. Biomimetic injectable hydrogel based on silk fibroin/hyaluronic acid embedded with methylprednisolone for cartilage regeneration. *Colloids Surf. B Biointerfaces* **2022**, *219*, 112859. [CrossRef] [PubMed]
35. Zhang, X.; Lian, R.; Fan, B.; Meng, L.; Zhang, P.; Zhang, Y.; Sun, W. ROS/enzyme dual-responsive drug delivery system for targeted colorectal cancer therapy: Synergistic chemotherapy, anti-inflammatory, and gut microbiota modulation. *Pharmaceutics* **2025**, *17*, 940. [CrossRef] [PubMed]
36. Su, X.Z.; Qiao, E.Q.; Teng, G.J.; Xiong, F. Quaternary ammonium salt microspheres loaded with vascular disrupting agents for targeted interventional therapy of hepatocellular carcinoma. *Acta Biomater.* **2025**, *203*, 591–603. [CrossRef]
37. Deng, Y.; Deng, X.; Li, Y.; Tian, J.; Wu, M.; Tang, J.; Liang, X.; Yang, X.; He, X.; Liu, Y.; et al. Size-adjustable nanoparticles co-target macrophages and endothelial cells for enhanced atherosclerosis therapy. *Colloids Surf. B Biointerfaces* **2025**, *255*, 114952. [CrossRef]
38. Teora, S.P.; Panavaité, E.; Sun, M.; Kiffen, B.; Wilson, D.A. Anisotropic, Hydrogel Microparticles as pH-Responsive Drug Carriers for Oral Administration of 5-FU. *Pharmaceutics* **2023**, *15*, 1380. [CrossRef] [PubMed]
39. Qian, D.; Xu, J.; Zhang, X.; Hu, F.; Cao, S.; Dong, Y.; Liu, X.; Yao, Y.; Yu, H.; Lu, Y.; et al. Microenvironment self-adaptive nanomedicine promotes spinal cord repair by suppressing inflammation cascade and neural apoptosis. *Adv. Mater.* **2024**, *36*, e2307624. [CrossRef] [PubMed]
40. Yang, Z.; Zhao, Y.; Zhang, X.; Huang, L.; Wang, K.; Sun, J.; Chen, N.; Yin, W.; Chen, S.; Zhi, H.; et al. Nano-mechanical immunoengineering: Nanoparticle elasticity reprograms tumor-associated macrophages via Piezo1. *ACS Nano* **2024**, *18*, 21221–21235. [CrossRef]
41. Zhang, W.; Liu, Y.; Wang, Z.; He, S.; Liu, W.; Wu, Y.; Yang, L.; Hu, C.; Wang, Y. Remodeling brain pathological microenvironment to lessen cerebral ischemia injury by multifunctional injectable hydrogels. *J. Control. Release* **2024**, *369*, 591–603. [CrossRef]
42. Gui, L.; Chen, Y.; Diao, Y.; Chen, Z.; Duan, J.; Liang, X.; Li, H.; Liu, K.; Miao, Y.; Gao, Q.; et al. ROS-responsive nanoparticle-mediated delivery of CYP2J2 gene for therapeutic angiogenesis in severe hindlimb ischemia. *Mater. Today Bio* **2021**, *13*, 100192. [CrossRef]
43. Chen, X.; Wu, Y.; Song, P.; Feng, L.; Zhou, Y.; Shi, J.; Dong, N.; Qiao, W. Matrix metalloproteinase-responsive controlled release of self-assembly nanoparticles accelerates heart valve regeneration in situ by orchestrating immunomodulation. *Adv. Sci.* **2025**, *12*, e2403351. [CrossRef]
44. Sun, X.; Yu, K.; Zhou, Y.; Dong, S.; Hu, W.; Sun, Y.; Li, Y.; Xie, J.; Lee, R.J.; Sun, F.; et al. Self-assembled pH-sensitive polymeric nanoparticles for the inflammation-targeted delivery of Cu/Zn-superoxide dismutase. *ACS Appl. Mater. Interfaces* **2021**, *13*, 18152–18164. [CrossRef]
45. Jia, Y.; Kong, X.; Li, R.; Wang, H.; Li, C.; Cheng, S.; Duan, W.; Xiao, Y.; Mai, Y.; Deng, W.; et al. Enhanced nasal-to-brain drug delivery by multivalent bioadhesive nanoparticle clusters for cerebral ischemic reperfusion injury protection. *Acta Biomater.* **2025**, *194*, 411–427. [CrossRef]
46. Guo, W.; Hu, C.; Wang, Y.; Zhang, W.; Zhang, S.; Peng, J.; Wang, Y.; Wu, J. NO-releasing double-crosslinked responsive hydrogels accelerate the treatment and repair of ischemic stroke. *Acta Pharm. Sin. B* **2025**, *15*, 1112–1125. [CrossRef]
47. Li, M.; Wang, Y.; Han, X.; Liu, Y.; Ma, M.; Zhang, L. Multifunctional polydopamine-based nanoparticles for dual-mode imaging-guided targeted therapy of lupus nephritis. *Pharmaceutics* **2022**, *14*, 1988. [CrossRef]
48. Fu, L.; Huo, S.; Lin, P.; Wang, J.; Zhao, J.; You, Y.; Nie, X.; Ding, S. Precise antibiotic delivery to the lung infection microenvironment boosts the treatment of pneumonia with decreased gut dysbiosis. *Acta Biomater.* **2024**, *184*, 352–367. [CrossRef]
49. Wang, S.; Zhai, Y.; Liu, M.; Cheng, Z.; Zhang, J.; Zhang, H.; Wang, H.; Hu, Y.; Li, C.; Luo, Z. A hydrogel-based drug delivery system reduces inflammation and oxidative stress to alleviate intervertebral disc degeneration. *Acta Biomater.* **2025**, *203*, 229–244. [CrossRef]
50. Hales, D.; Muntean, D.M.; Neag, M.A.; Kiss, B.; Ștefan, M.G.; Tefas, L.R.; Tomuță, I.; Sesărman, A.; Rațiu, I.A.; Porfire, A. Curcumin-loaded microspheres are effective in preventing oxidative stress and intestinal inflammatory abnormalities in experimental ulcerative colitis in rats. *Molecules* **2022**, *27*, 5680. [CrossRef]
51. Zeng, Q.; Gong, Y.; Jiao, W.; Xu, J.; Chen, X.; Xu, R.; Liu, Y.; Liang, X.; Li, G.; Liu, J.; et al. Smart ultra-long-lasting sequentially triggerable and artfully implantable nozzle system for on-demand drug delivery for chronotherapy. *Sci. Adv.* **2025**, *11*, eadv8734. [CrossRef]
52. Cheng, X.; Yu, P.; Zhou, X.; Zhu, J.; Han, Y.; Zhang, C.; Kong, L. Enhanced tumor homing of pathogen-mimicking liposomes driven by R848 stimulation: A new platform for synergistic oncology therapy. *Acta Pharm. Sin. B* **2022**, *12*, 924–938. [CrossRef]
53. Wang, L.; Peng, Y.; Song, L.; Xia, D.; Li, C.; Li, Z.; Li, Q.; Yu, A.; Lu, C.; Wang, Y. Colchicine-containing nanoparticles attenuate acute myocardial infarction injury by inhibiting inflammation. *Cardiovasc. Drugs Ther.* **2022**, *36*, 1075–1089. [CrossRef]

54. Wang, Y.; Zhang, D.; Jia, M.; Zheng, X.; Liu, Y.; Wang, C.; Lei, F.; Niu, H.; Li, C. ZIF-8 nanoparticles coated with macrophage-derived microvesicles for sustained, targeted delivery of dexamethasone to arthritic joints. *J. Drug Target.* **2022**, *30*, 1006–1016. [CrossRef]
55. Yang, Z.; Cao, F.; Li, H.; He, S.; Zhao, T.; Deng, H.; Li, J.; Sun, Z.; Hao, C.; Xu, J.; et al. Microenvironmentally optimized 3D-printed TGFβ-functionalized scaffolds facilitate endogenous cartilage regeneration in sheep. *Acta Biomater.* **2022**, *150*, 181–198. [CrossRef]
56. Hu, C.; Qiu, Y.; Guo, J.; Cao, Y.; Li, D.; Du, Y. An oxygen supply strategy for sonodynamic therapy in tuberculous granuloma lesions using a catalase-loaded nanoplatform. *Int. J. Nanomed.* **2023**, *18*, 6257–6274. [CrossRef]
57. Liu, Y.; He, J.; Li, M.; Ren, K.; Zhao, Z. Inflammation-driven nanohitchhiker enhances postoperative immunotherapy by alleviating prostaglandin E2-mediated immunosuppression. *ACS Appl. Mater. Interfaces* **2024**, *16*, 6879–6893. [CrossRef]
58. Yuan, L.; Yao, L.; Ren, X.; Chen, X.; Li, X.; Xu, Y.; Jin, T. Cartilage defect repair in a rat model via a nanocomposite hydrogel loaded with melatonin-loaded gelatin nanofibers and menstrual blood stem cells: An in vitro and in vivo study. *J. Mater. Sci. Mater. Med.* **2024**, *35*, 55. [CrossRef] [PubMed]
59. Tan, X.; Zhou, Y.; Qin, Y.; Wu, L.; Yang, R.; Bao, X.; Jiang, R.; Sun, X.; Ying, X.; Ben, Z.; et al. Self-healing hydrogel resulting from the noncovalent interaction between ropivacaine and low-molecular-weight gelator sodium deoxycholate achieves stable and endurable local analgesia in vivo. *ACS Appl. Mater. Interfaces* **2024**, *16*, 45969–45988. [CrossRef] [PubMed]
60. Qin, X.; Zhu, L.; Zhong, Y.; Wang, Y.; Wu, G.; Qiu, J.; Wang, G.; Qu, K.; Zhang, K.; Wu, W. Spontaneously right-side-out-orientated coupling-driven ROS-sensitive nanoparticles on cell membrane inner leaflet for efficient renovation in vascular endothelial injury. *Adv. Sci.* **2023**, *10*, e2205093. [CrossRef] [PubMed]
61. Wei, B.; Chen, L.; Huang, X.; Chi, F.; Li, G.; Yang, L.; Zheng, C.; Wang, Y. Microenvironment-responsive biomimetic bioprosthetic valve with antithrombosis and immunoregulation performance. *ACS Appl. Mater. Interfaces* **2025**, *17*, 18160–18178. [CrossRef]
62. Shen, M.; Yao, S.; Li, S.; Wu, X.; Liu, S.; Yang, Q.; Du, J.; Wang, J.; Zheng, X.; Li, Y. A ROS- and shear stress dual-sensitive bionic system with cross-linked dendrimers for atherosclerosis therapy. *Nanoscale* **2021**, *13*, 20013–20027. [CrossRef] [PubMed]
63. Jori, C.; Ahmad, A.; Kumar, A.; Kumar, B.; Ali, A.; Ali, N.; Tabassum, H.; Khan, R. Bioactive chitosan-BSA Maillard-derived chrysin-loaded nanoparticles: A gastroprotective, biomucoadhesive approach for enhanced oral therapy in ulcerative colitis. *Carbohydr. Polym.* **2025**, *359*, 123537. [CrossRef] [PubMed]
64. Wang, C.; Xiao, Z.; Fan, J.; Zhang, C.; Wang, T.; Qiu, Z.; Ye, F.; Chen, M.; Li, Y. Nanocarriers loaded with danshensu for treating ischemic stroke by reducing oxidative stress and glial overactivation. *ACS Omega* **2024**, *9*, 35686–35694. [CrossRef]
65. Wang, D.; Sun, M.; Zhang, Y.; Chen, Z.; Zang, S.; Li, G.; Li, G.; Clark, A.R.; Huang, J.; Si, L. Enhanced therapeutic efficacy of a novel colon-specific nanosystem loading emodin on DSS-induced experimental colitis. *Phytomedicine* **2020**, *78*, 153293. [CrossRef]
66. Hu, D.; Huang, Z.; Li, W.; Shan, L.; Wu, M.Y.; Feng, S.; Wan, Y. Macrophage membrane-cloaked ROS-responsive albumin nanoplatforms for targeted delivery of curcumin to alleviate acute liver injury. *Mol. Pharm.* **2025**, *22*, 771–786. [CrossRef]
67. Hsu, C.Y.; Lin, J.; Wei, M.F.; Chen, L.H.; Liang, H.T.; Lin, F.H. Local delivery of carboplatin-loaded hydrogel and calcium carbonate enables two-stage drug release for limited-dose radiation to eliminate mouse malignant glioma. *Biomaterials* **2025**, *312*, 122746. [CrossRef]
68. Chen, Y.W.; He, A.C.; Huang, T.Y.; Lai, D.H.; Wang, Y.P.; Liu, W.W.; Kuo, W.T.; Hou, H.H.; Cheng, S.J.; Lee, C.Y.; et al. Iontophoresis-enhanced buccal delivery of cisplatin-encapsulated chitosan nanoparticles for treating oral cancer in a mouse model. *Int. J. Nanomed.* **2024**, *19*, 10435–10453. [CrossRef]
69. Qiu, X.; Yu, Y.; Liu, H.; Li, X.; Sun, W.; Wu, W.; Liu, C.; Miao, L. Remodeling the periodontitis microenvironment for osteogenesis by using a reactive oxygen species-cleavable nanoplatform. *Acta Biomater.* **2021**, *135*, 593–605. [CrossRef] [PubMed]
70. Mudigonda, J.; Onohara, D.; Amedi, A.; Suresh, K.S.; Kono, T.; Corporan, D.; Padala, M. In vivo efficacy of a polymer-layered decellularized matrix composite as a cell-homing cardiovascular tissue substitute. *Mater. Today Bio* **2022**, *17*, 100451. [CrossRef]
71. Zhang, L.; Lu, K.; Cao, Y.; Cui, L.; Li, X.; Guo, P.; Fu, H.; Zhu, C.H. An injectable dual-layer chitosan-alginate nano-hydrogel incorporated Cu(I)-isopolymolybdate-based metal-organic framework tailored for three-in-one anti-cancer nanotherapy. *Int. J. Biol. Macromol.* **2024**, *282*, 137381. [CrossRef] [PubMed]
72. Abbas, S.M.; Sudheer, P. Development and evaluation of curcumin-Eudragit microsponges for anti-inflammatory therapy of the colon. *Bull. Pharm. Sci. Assiut. Univ.* **2024**, *47*, 789–803. [CrossRef]
73. Wang, Q.Y.; Xu, Y.S.; Zhang, N.X.; Dong, Z.P.; Zhao, B.N.; Liu, L.C.; Lu, T.; Wang, Y. Phenylboronic ester-modified anionic micelles for ROS-stimuli response in HeLa cells. *Drug Deliv.* **2020**, *27*, 681–690. [CrossRef] [PubMed]
74. Maleki, H.; Doostan, M.; Farzaei, M.H.; Seifi, P.; Miraghaee, S.; Doostan, M. Achillea wilhelmsii-incorporated chitosan@Eudragit nanoparticles intended for enhanced ulcerative colitis treatment. *AAPS PharmSciTech* **2023**, *24*, 112. [CrossRef]
75. Jin, L.; Zhu, Z.; Hong, L.; Qian, Z.; Wang, F.; Mao, Z. ROS-responsive 18β-glycyrrhetic acid-conjugated polymeric nanoparticles mediate neuroprotection in ischemic stroke through HMGB1 inhibition and microglia polarization regulation. *Bioact. Mater.* **2022**, *19*, 38–49. [CrossRef] [PubMed]

76. Yipel, M.; Tekeli, İ.O.; Altinok-Yipel, F.; Coşkun, P.; Aslan, A.; Güvenç, M.; Beyazit, N.; Özsoy, Ş.Y. The protective effect of boswellic acid- and ellagic acid-loaded, colon-targeted, pH-sensitive N-succinyl chitosan in an ulcerative colitis rat model. *J. Drug Deliv. Sci. Technol.* **2022**, *68*, 103023. [CrossRef]
77. Zhang, Y.; Zhang, H.; Zhao, F.; Jiang, Z.; Cui, Y.; Ou, M.; Mei, L.; Wang, Q. Mitochondrial-targeted and ROS-responsive nanocarrier via nose-to-brain pathway for ischemic stroke treatment. *Acta Pharm. Sin. B* **2023**, *13*, 5107–5120. [CrossRef]
78. Fan, S.; Zhao, Y.; Yao, Y.; Shen, X.; Chai, X.; Li, J.; Pi, J.; Huang, X.; Jin, H.; Zhou, Z. Oral colon-targeted pH-responsive polymeric nanoparticles loading naringin for enhanced ulcerative colitis therapy. *J. Transl. Med.* **2024**, *22*, 878. [CrossRef]
79. Zhen, P.; Jiang, Q.; Yu, F.; Xu, X.; Wei, Q.; Liu, X.; Sun, X.; Liang, G.; Tong, J. ROS-differentiated release of Apelin-13 from hydrogel comprehensively treats myocardial ischemia-reperfusion injury. *J. Control Release* **2025**, *379*, 609–620. [CrossRef] [PubMed]

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SHORT COMMUNICATION

Major and trace elements in whole blood of phlebotomized patients with porphyria cutanea tarda

Mariann Dinya[a], E. Székely[b], K. Szentmihályi[c], Gy. Tasnádi[b], A. Blázovics[d]

[a]*Department of Pharmaceutics, Faculty of Pharmacy, Semmelweis University, Hőgyes Endre u. 7, H-1092 Budapest, Hungary*
[b]*Hungarian Porphyria Center, Central Hospital of the Hungarian State Railways, Budapest, Hungary*
[c]*Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary*
[d]*Second Department of Medicine, Semmelweis University, Budapest, Hungary*

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Abstract

Porphyria cutanea tarda (PCT) is a disorder of hem biosynthesis resulting from a decreased activity of the uroporphyrinogen decarboxylase enzyme. Hem precursors are accumulated in the blood, liver and skin. Inherited and acquired factors also contribute to the pathogenesis of PCT. Hem precursors and porphyrins are excreted with urine and faeces.

Whole blood of 8 PCT patients and 6 volunteers of Caucasian origin were analysed. In addition to routine laboratory measurements, 19 elements (Al, B, Ba, Ca, Cd, Co, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, S, V, Zn) were determined by means of inductively coupled plasma optical emission spectrometry (ICP-OES).

Mg, P and S concentrations in whole blood were decreased significantly ($p<0.05$), whereas Ba was increased in PCT patients compared to controls. Metabolic alterations are reflected in the correlation of parameters. Positive correlations were found between the element pairs of Zn–Al, Zn–Mg, Zn–Mn, B–S, Fe–Mg, K–P, Mg–Mn for PCT patients, whereas in the control group Al–Mn, Ca–Cu, Ca–Na, Cu–Mg, Fe–K, Mg–Na, Zn–P showed positive correlations.

Keywords: Porphyria cutanea tarda (PCT); Major and trace elements; ICP-OES

Introduction

Porphyria cutanea tarda (PCT) is a common form of porphyria. PCT is differentiated from porphyrias by clinical (skin lesion) and biochemical (high uroporphyrin (URO) level in blood, urine and faeces) parameters. PCT is a human metabolic disorder, determined by acquired or genetic factors. This disease is characterized by the strongly decreased activity of the enzyme uroporphyrinogen decarboxylase (UROD), and the accumulation of UROs and heptacarboxylporphyrin mainly in the liver and skin [1]. Erythrocyte UROD activity is normal in the sporadic form of PCT. UROD activity was found to be reduced in tissues (skin, erythrocytes, liver) in familial PCT. The enzyme defect is inherited as autosomal dominant. Alcohol consumption, virus infections, hepatotoxic chemicals, hepatic siderosis, estrogens and drug abuse determine clinical manifestations of PCT [2,3].

Excess URO production in the liver associated with consumption of alcohol and moderate iron overload causes skin lesions in the PCT patients. The lesions

E-mail address: m_dinya@yahoo.com (M. Dinya).

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are more pronounced in summer and in sunny climates [4].

Excessive porphyrinuria with dominance of URO is one of the indicators in PCT. [6]. Therapeutic phlebotomy reduces the iron load and the UROs [5]. In most cases, PCT is associated with high iron levels in the liver; and the excess iron may cause decreased activity of UROD. Transferrin receptor polymorphism may influence the affinity of this receptor to its ligand with a subsequent increase of cellular ion absorption and storage [6]. The resolution of the cutaneous manifestations and porphyrin overproduction with venesection is associated with the normalization of hepatic UROD activity in sporadic PCT [7].

Element concentrations in the blood of phlebotomized PCT patients have not been determined so far. Therefore, the concentrations of 19 major and trace elements were determined in whole blood samples of PCT patients.

Materials and methods

Materials

Alcohol and acetic acid were purchased from Merck (Darmstadt, Germany). The ''Porphyrin Acids Marker Kit'' was from Porphyrin Products Inc. All other chemicals were of the highest purity grade available and were obtained from Reanal, Budapest.

Subjects and samples

Eight male PCT patients of Caucasian origin (average age: 62 ± 13 years) were examined. The diagnosis of PCT was based on the results of blood and urine analyses. The control group consisted of 6 male Caucasian volunteers (average age: 43 ± 13 years).

Routine clinical parameters were determined in serum directly after the sampling of blood. Determinations in urine were carried out in urine collected within 24 h. Whole blood samples were kept in a freezer ($-20\,^{\circ}C$) until sample preparation for inductively coupled plasma optical emission spectrometry (ICP-OES) measurements.

All procedures were in accordance with the ethical standards formulated in the Helsinki Declaration of 1975 (revised 1983), permission number: IKEB 5/2003.

Clinical parameters

Routine clinical parameters were measured by using an Olympus AU-600 and a Roche/Hitachi MODULAR instrument (nr = normal range): aspartate aminotransferase (AST) (nr: 0–35 U/L), alanine aminotransferase (ALT) (nr: 0–43 U/L), gamma-glutamyltransferase (GGT) (nr: 7–47 U/L), and alkaline phosphatase (ALP) (nr: 98–279 U/L) enzyme activities, serum triglycerides (TG) (nr: 0.6–1.7 mmol/L), high-density lipoprotein (HDL-CHOL) (nr: >1.45 mmol/L), low-density lipoprotein (LDL-CHOL) (nr: <4.0 mmol/L), cholesterol (CHOL) (nr: 3.2–5.2 mmol/L), iron (nr: 6.0–27.0 μmol/L), transferrin (nr: 2.1–3.8 g/L), ferritin (nr: 30–400 ng/m). Hemoglobin (HGB) (nr: 13.5–17.5 g/dL) was determined with a Cell-dyn Haematological Automate. The Laboratory parameters were measured with Randox (Ardmore, UK) and Roche (Mannheim, Germany) kits.

Urinary porphyrin

Urine was collected and kept in dark bottles for 24 h at $4\,^{\circ}C$. The concentration of uro- and coproporphyrins was measured by the method of Westerlund [8].

Sample preparation

For element determinations, the whole blood samples (weight ranges: 1.5–2.0 g) were digested with a mixture of HNO_3 (7 mL) and H_2O_2 (2 mL) in Parr bombs. After digestion, the samples were diluted to 25 mL with deionized water [9].

Element determinations

Since most elements are intracellular, serum samples do not show the relevant element status. Therefore element concentrations were determined in whole blood by ICP-OES with an Atom Scan25 (Thermo Jarrell Ash) instrument, a sequential spectrometer. The following 19 elements were determined: aluminium (Al), boron (B), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), phosphorous (P), sulfur (S), vanadium (V), zinc (Zn).

Three times 3 s integration time, blank subtraction and background correction were applied during the measurements. The working solutions for calibration were made by diluting ICP standards from Merck (Darmstadt, Germany).

The method was optimized for all elements and the recovery was between 90.6% (phosphorus) and 116.2% (boron) [10,11].

Statistical analysis

Summary statistics (n, mean, standard deviation, correlation coefficient) were calculated. The changes between control and patient groups were analysed using

t-test. A probability value of 0.05 or less was considered significant. Statistical analysis was performed with STATISTICA 6.0 software.

Results

Tables 1–3 show the clinical parameters for PCT patients and controls. Most of the parameters did not differ significantly between the PCT and the control group. The amount of porphyrins in urine increased, the main component was URO. Twenty-four hours urinary porphyrins were between 45 and 500 nmol.

The evaluation of the data showed that serum Fe concentrations were changed together with ALT ($r = 0.84$) in the phlebotomized PCT patients ($p < 0.05$) but not in the control group.

The detection limits and results of element determination in whole blood of patients with PCT are shown in Table 4. The detection limits were calculated from the detection limits of the instrument, the weight of the blood samples and the final volumes. The concentrations of Cd, Co, Li, Mo, Ni and V were below detection limit, therefore, they were omitted from the table.

Blood Ba, Al, B and Ca levels in the PCT group were increased compared to the control group, and the Al and B levels in the PCT group were also higher compared to normal values. In the case of Mn, Zn, P, Mg, K and S, the results were below the concentrations determined in the control group and normal values.

The blood element concentrations from PCT patients were compared statistically with those of the controls. Significant differences ($p < 0.05$) between the groups were observed for the elements Ba, B, Mg, P and S.

Since the metal ion metabolism is changed in the disease, the correlations between different elements are also altered. The exception is the correlation between Al–Mn ($r = 0.96$; 0.99) in both the control and the phlebotomized PCT group. Positive correlations were found in phlebotomized PCT patients for Al–Mg ($r = 0.89$), Al–Zn ($r = 0.77$), B–S ($r = 0.84$), Fe–Mg ($r = 0.79$), K–P ($r = 0.88$), Mg–Mn ($r = 0.87$), Mn–Zn ($r = 0.78$), Mg–Zn ($r = 0.78$) and in controls for Ca–Cu ($r = 0.83$), Ca–Na ($r = 0.86$), Cu–Mg ($r = 0.90$), Fe–K ($r = 0.85$), Na–Mg ($r = 0.86$), Zn–P ($r = 0.94$).

Table 1. Liver parameters (mean ± SD) of porphyria cutanea tarda (PCT) patients compared to the control group

	PCT ($n = 8$)	Controls ($n = 6$)
AST (U/L)	36.25 ± 16.73	29.66 ± 4.96
ALT (U/L)	36.50 ± 26.54	21.00 ± 7.32
GGT (U/L)	50.50 ± 21.91	24.00 ± 9.26
ALP (U/L)	230.00 ± 101.60	161.83 ± 52.30

AST, aspartate-aminotransferase; ALT, alanine-aminotransferase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase.

Table 2. Lipid parameters (mean ± SD) of porphyria cutanea tarda (PCT) patients compared to the control group

	PCT ($n = 8$)	Controls ($n = 6$)
CHOL (mmol/L)	4.48 ± 1.04	4.42 ± 0.30
TG (mmol/L)	1.03 ± 0.44	1.02 ± 0.43
LDL-CHOL (mmol/L)	2.69 ± 0.82	2.39 ± 0.35
HDL-CHOL (mmol/L)	1.31 ± 0.54	1.39 ± 0.35

CHOL, cholesterol; TG, triglycerides; LDL-CHOL, low density lipoprotein; HDL-CHOL, high density lipoprotein.

Table 3. Iron metabolism parameters (mean ± SD) of porphyria cutanea tarda (PCT) patients compared to the control group

	PCT ($n = 8$)	Controls ($n = 6$)
HGB (g/dL)	15.22 ± 1.77	14.37 ± 26.05
Iron (μmol/L)	27.83 ± 8.06	20.05 ± 8.99
Transferrin (g/L)	2.43 ± 0.31	2.78 ± 0.15
Ferritin (ng/mL)	150.05 ± 123.99	76.41 ± 13.03

HGB, hemoglobin.

Table 4. Results of element determinations (mean ± SD) in whole blood of phlebotomised PCT patients and controls as well as detection limits

	Control ($n = 6$) (μg/g)	PCT ($n = 8$) (μg/g)	Detection limit (μg/g)
Al	0.658 ± 0.313	2.748 ± 1.169	0.1
B	0.657 ± 0.493	2.402 ± 1.04	0.02
Ba	0.013 ± 0.005	0.108 ± 0.048*	0.002
Ca	53.64 ± 11.89	66.41 ± 26.79	0.002
Cu	0.738 ± 0.159	0.706 ± 0.130	0.01
Fe	530.4 ± 51.7	483.82 ± 51.18	0.01
K	1790 ± 183	1542.74 ± 225.06	0.5
Mg	41.37 ± 6.15	32.52 ± 5.96*	0.0025
Mn	0.105 ± 0.071	0.039 ± 0.015	0.005
Na	2323.00 ± 160.00	2270.01 ± 139.09	0.05
P	329.60 ± 18.31	297.22 ± 24.48*	0.25
S	1501 ± 83	1319.70 ± 145.49*	0.25
Zn	7.85 ± 0.99	6.79 ± 1.78	0.001

*Significant difference vs. controls.

Discussion and conclusions

Mainly URO accumulation causes the photodermatosis and Fe overload increases the lipid peroxidation in cutaneous symptoms. The lower concentration of Zn

and the significantly low concentrations of Mg, S and P in whole blood may contribute to the cutaneous reaction – skin fragility, blisters on the area exposed to the sun – in PCT; therefore further investigations are required to elucidate the relations between the elements.

The concentrations of Al and B are relatively high in PCT compared to the control and the normal values (n.r. $< 0.2\,\mu g/g$). Although the Ba concentration was significantly higher in PCT patients than in the controls, this concentration is similar to the normal value.

Correlations were found between the concentrations of Al and Mn ($r = 0.96$; 0.99) both in the control and the phlebotomized PCT group, whereas the other element correlations in the control group changed. Similarly, altered correlations were found in other diseases, e.g. in IBD, in which totally different correlations were observed, e.g. positive correlations for Al–Cu and Fe–Cu [12]. The similarity of the two diseases is the positive correlation between the concentrations of Al and Zn, and of Fe and Mg.

Phlebotomy, although having been proved to be the proper treatment supported by the clinical data, does not modify efficiently the metal-ion components in the whole blood of the patients.

Acknowledgements

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References

[1] Siersema PD, Van Helvoirt RP, Cleton-Soeteman MI, De Bruijn WC, Wilson JHP, Van Eijk HG. The role of iron in experimental porphyria and porphyria cutanea tarda. Biol Trace Elem Res 1992;35:65–72.

[2] Hindmars JT, Oliveras L, Greenway DC. Biochemical differentiation of the porphyrias. Clin Biochem 1999;32:609–19.

[3] Tavazzi D, Martinez di Montemuros F, Fargion S, Fracanzani AL, Fiorelli G, Cappellini MD. Levels of uroporphyrinogen decarboxylase (URO-D) in erythrocytes of Italian porphyria cutanea tarda patients. Cell Mol Biol 2002;48:27–32.

[4] Doss MO, Kuhnel A, Gross U. Alcohol and porphyrin metabolism. Clin Biochem 2000;35-2:109–25.

[5] Bruce AJ, Ahmed I. Childhood-onset porphyria cutanea tarda: successful therapy with low-dose hydroxychloroquine (Plaquenil). J Am Acad Dermatol 1998;38:810–4.

[6] Dereure O, Aquilar-Martinez P, Bessis D, Perney P, Vallat C, Guillot B, et al. HFE mutations and transferrin receptor polymorphism analysis in porphyria cutanea tarda: a prospective study of 36 cases from southern France. Br J Dermatol 2001;144:533–9.

[7] Elder GH, Urquhart AJ, De Salamanca R. Immunoreactive uroporphyrinogen decarboxylase in the liver in porphyria cutanea tarda. Lancet 1985;i:229–32.

[8] Westerlund J, Pudek M, Schreiber WE. Principles and techniques. Clin Chem 1988;34:345–55.

[9] Szentmihályi K, Sípos P, Blázovics A, Vinkler P, Szilágyi M. Concentration of biliary metal elements and gallstone formation in humans (cholelithiasis). Trace Elem Electrol 2002;19:160–4.

[10] Szentmihályi K, Csiktusnádi-Kiss GA, Keszler À, Kótai L, Candeaias M, Bronze MR, et al. Method development for measurement of elements in Hungarian red wines by inductively coupled plasma optical emission spectrometry (ICP-OES). Acta Alimentaria 2000;29:105–21.

[11] Szentmihályi K, Fehér E, Vinkler P, Kéry À, Blázovics A. Metabolic alteration of toxic and nonessential elements by the treatment of *Sempervivum tectorium* extract in a hyperlipidemic rat model. Toxicol Pathol 2004;1:50–7.

[12] Blázovics A, Szentmihályi K, Vinkler P, Kovács À. Zn overdose may cause disturbance in the iron metabolism. Trace Elem Electrol 2004;21(4):240–7.

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Effect of inhibitors of myeloperoxidase on the development of aortic atherosclerosis in an animal model

Gábor Békési[a,*], Helmut Heinle[b], Réka Kakucs[a], Tamás Pázmány[c], Dezső Szombath[d], Mariann Dinya[e], Zsolt Tulassay[a], János Fehér[a], Károly Rácz[a], Béla Székács[a], Éva Riss[f], Andrea Farkas[g], Ferenc Gódor[f], György Illyés[g]

[a]*2nd Department of Medicine, Semmelweis University, Szentkirályi utca 46, 1088 Budapest, Hungary*
[b]*Institute of Physiology, Eberhard-Karls University, Gmelin Str. 5, 72076 Tuebingen, Germany*
[c]*Department of Immunology, Roswell Institute, Elm and Carlton Street, Buffalo, NY 14263, USA*
[d]*Institute of Pathophysiology, Semmelweis University, Nagyvárad tér 4, 1089 Budapest, Hungary*
[e]*Institute of Pharmacotechnology, Semmelweis University, Hőgyes E. utca 7, 1092 Budapest, Hungary*
[f]*Maecenator Foundation, Budapest, Erzsébet krt. 13, 1073 Budapest, Hungary*
[g]*2nd Department of Pathology, Semmelweis University, Üllői út 93, 1082 Budapest, Hungary*

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Abstract

Our earlier studies have shown that some steroids increase myeloperoxidase enzyme (MPO) release from human granulocytes, and that MPO plasma levels are significantly lower in postclimacteric people. Moreover, we have proven that MPO inhibits production of atherogenic free radical superoxide anion and MPO-inhibitors increase superoxide release. The aim of the present study was to investigate the effect of MPO-inhibitors on the early phase of aortic atherosclerosis, namely the extent of intimal plaques and the thickening of the medial layer. Adult male rabbits were fed with lipid rich food (cholesterol: 1.3%, peanut oil: 8%) for 8 weeks. During this period MPO-inhibitors were also given (4-aminobenzoicacid-hydrazide/ABAH/-13.3 mg/kg/day or indometacin-5 mg/kg/day). All animals developed intimal lipid plaques (raised fatty streaks). The relative plaque-covered areas of the aortas were compared and the media thickness of the aorta was measured on plaque-free as well as plaque-containing areas. The medial smooth muscle density and peroxidase activity of the aortic media were also determined. The media thickness increased ($p<0.05$) in the cholesterol + ABAH as well as in the cholesterol + indometacin groups up to 375.7 ($\pm$60.5) and 442.5 ($\pm$123.4) μm, respectively, compared to the control group (cholesterol feeding alone) where it measured only 308.4 ($\pm$51.67) μm. The medial peroxidase activity decreased significantly in the indometacin treated group and showed a decreasing tendency using ABAH. In parallel to this there was a tendency of increase in the relative plaque covered areas. The smooth muscle density showed no significant modifications, while inhibitors of the MPO seemed to enhance aortic medial thickness, i.e. the grade of a pre-atherosclerotic lesion, in our animal model. Collectively, the anti-atherogenic effect of certain steroid hormones might be realized through the impact on MPO activity.

Keywords: Myeloperoxidase; Myeloperoxidase inhibitors; Cholesterol fed rabbits; Media thickness; Atherosclerosis

1. Introduction

The degradation of toxic byproducts of aerobic respiration produces excess hydrogen peroxide (H2O2), which is, in turn, destroyed by heme proteins known as peroxidases. Peroxidase reduces H2O2 to water while oxidizing a variety of substrates. Myeloperoxidase is the best caracterized enzyme in animal and human tissues, it is abundantly secreted from activated phagocytes. MPO catalyzes the oxidation of halide ions to hypohalous acids at the expense of hydrogen peroxide. Other MPO—like enzymes, possessing a peroxidase activity are widely distributed in different tissues. The biological consequences of these reactions in vivo are still unclear.

* Corresponding author. Tel.: +36 1 317 4548; fax: +36 1 266 0816.
E-mail address: bekesig@invitel.hu (G. Békési).

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In most of the previous publications the higher level of MPO was connected to enhanced free radical production and greater exposure to atherosclerosis, thus, it has been known to possess a prooxidant, i.e. atherogenic ability (Mohácsi et al., 1996; Dougherty et al., 1994). The presence of the enzyme in atherosclerotic lesions also backs up this view (Dougherthy et al., 1994; Heinecke, 2002).

However, other authors argue that increased MPO activity also contributes to the termination of the free radical production through inactivation of NADPH-oxidase enzyme, generating superoxide anion (Jandl et al., 1978; cited by Feher et al., 1987).

Some in vitro and in vivo data ascribing a significant role for certain steroids in the regulation of granulocyte MPO enzyme activity and release are already known. Estrogen had been demonstrated to enhance the myeloperoxidase activity in human polymorphonuclear leukocytes (Jansson, 1990). Estrogen replacement therapy in postmenopausal women also increased MPO activity and release from neutrophils (Bekesi et al., 1997). Testosterone and prednisolone treatment seemed to have such an effect, too (Bekesi et al., 2001a). We also proved a physiologically decreasing-with-age plasma concentration of MPO in both genders (Bekesi et al., 2001a).

Certain steroids show superoxide inhibiting capacity (Bekesi et al., 2000). However, other steroid compounds do not have such a property (Bekesi et al., 2004). MPO inhibitors abolished even the steroidal antioxidant effect (Bekesi, 2001a).

Oxygen derived free radicals such as superoxide anion are responsible for LDL-oxidation, the highly important key factor in atherogenesis (Babior, 2000). Although the free radical superoxide anion is reactive only to a limited degree, it also induces peroxidatic changes in lecithin-cholesterol membranes (Seligman et al., 1979) and can be transformed into other more reactive oxygen intermediates, so it is of great importance. By cooperating with hydrogen peroxide, superoxide anion can produce hydroxyl radical and singlet oxygen, which are the proximate causes of lipid peroxidation (Kellogg and Fridovich, 1977). Beyond the oxidative role of superoxide anion it is also a vasoconstrictor (Das, 1992) and significantly stimulates vascular smooth muscle cell proliferation in aortic explant of rats (Cathapermal et al., 1998). By neutralizing the vasodilator nitric oxide (NO) superoxide anion produces peroxynitrite, which also oxidizes the lipoproteins, decomposes to highly reactive species (Van Der Vliet et al., 1994) and contributes to the loss of cardiac efficiency in animal heart (Ferdinandy et al., 1999).

A plasma membrane-associated NADPH oxidase is present not only in neutrophils but also in endothelial cells, vascular smooth muscle cells and fibroblasts and seems to be the most important source of superoxide anion in the vessel wall (San José et al., 2004).

In a recent study we demonstrated that adding MPO to separated neutrophils results in decreased release of free radical superoxide anion from these cells (Bekesi et al., 2001b). MPO inhibitors, on the other hand, have been proven to enhance the superoxide anion production of neutrophil granulocytes (Bekesi et al., 2001a). MPO-deficient mice are known to develop severe atherosclerosis (Brennan et al., 2001a).

Returning to the previously mentioned harmful features of superoxide anion and to the superoxide inhibiting ability of certain steroids, the explanation is clear that estrogen, and perhaps other steroid compounds, are able to inhibit smooth muscle cell proliferation and thereby, among other things, temper with the extent of damage to the vascular wall. Hormone deficiency related to menopause, by means of the deficient steroidal effect—in our opinion also because of a reduction in the enhancing effect towards the MPO enzyme—may therefore justifiably lead to an increase in intima-media thickness providing a degree of pre-clinical atherosclerosis. Several statements published in this subject area conform to the contents of this chapter: early menopause is associated with increased sub-clinical atherosclerosis [intima-media thickness] (Mack et al, 2004). Several outstanding publications also deal with estrogen's anti-proliferating nature for smooth muscle cells (Dai-Do et al, 1996; Joswig et al, 1999; Espinosa et al, 1996). In the knowledge of inhibition by estrogen on production of superoxide anion, with its proliferating effect, this seems to have a biochemical basis.

4-Aminobenzoic acid hydrazide (ABAH) is a specific MPO inhibitor (Mytar et al., 1999). MPO oxidizes ABAH to a radical that reduces the enzyme to its ferrous intermediate. Ferrous MPO reacts either with oxygen to allow enzyme turnover, or with hydrogen peroxide to give irreversible inactivation (Kettle et al., 1997). Indomethacin has also been known to be a strong MPO inhibitor. It directly inhibits the chlorinating activity of the enzyme (Shacter et al., 1991).

Feeding rabbits with cholesterol-rich food for several weeks has been considered an appropriate method for developing atherosclerotic plaques in the vessel walls of the animals and for investigating the pathogenetic impact of free radicals on atherogenesis (Heinle, 2002). Ultrasonography has been applied recently to detect the early stage of atherosclerosis. The thickened intima-media is associated with an increased cardiovascular risk (Bots et al., 1999; Salonen et al., 1991; Kadar et al. 1999).

Accordingly, in the present study we planned to test the effect of MPO-inhibitors ABAH and indomethacin on aortic atherosclerosis and medial thickness. We used an experimentally induced atherosclerosis model in rabbits, which disclosed alterations corresponding to an early phase atherosclerosis in humans.

2. Methods

New-Zealand white male adult rabbits were fed with lipid rich food (cholesterol: 1.3%, peanut oil: 8%) for 8 weeks.

During this period the animals were also given orally administered MPO-inhibitors (4-aminobenzoicacid-hydrazide/ABAH/ 13.33 mg/kg/day, or indometacin 5 mg/kg/day) in parallel with the only cholesterol-fed control group ($n=5$/group). No data are available in the literature on the in vivo administration of ABAH. Effective concentrations used in our previous in vitro experiments were extrapolated to the extra-cellular volume of the animals. In vivo dosage of indomethacin, on the other hand, is well known and established in animal experiments. In terms of size, the quantity of all daily indomethacin given to our experimental animals is close to the maximum dose allowable therapeutically under human conditions (mg/kg/day).

2.1. Histochemical staining

Rabbits were killed by intravenously administered sodium pentobarbital overdose. The aorta was dissected and opened longitudinally from the end of the aortic roots to the iliac bifurcation, then pinned on boards and fixed in 4% buffered formalin.

The intimal plaques were stained red with Sudan stain for lipids, then the aortas were photographed using a digital camera (Nicon Coolpix 4500).

After this aortic segments were rolled up and embedded in OCT(Optimal Cutting Temperature Compound; 10.24% polyvinyl alcohol, 4.26% polyethylene glycol, 85.5% non-reactive ingredient; Miles Inc. Elkhart, IN, USA) then frozen at -22 °C. These specimens were sectioned 10 μm thick, proceeding deep enough so the entire length of the aorta segment could be viewed in a spiral configuration. This technique allows perpendicular cuts on longitudinally oriented aortas. For medial thickness and cell density measurements sections were stained with hematoxylin.

To measure the peroxidase activity, adjacent frozen tissue sections were further processed. Under a dissecting microscope aortic wall segments were dissected from these slides using sharp blades. All collected aortas were placed on a single, wet silane coated microscope slide (previously treated with a 2% solution of 3-aminopropyltriethoxy-silane in acetone; SIGMA A-3648) and dried. This allowed us to measure the peroxidase activity of all the collected aorta segments in a single assay (microarray technique). The peroxidase reaction was performed by immersing the slide in a diaminobenzidine tetrahydrochloride (DAB, VIEW Ventana, Tucson, AZ 85737) solution containing hydrogen peroxide for 20 min (10 ml TRIS buffer, pH 8.2; 6 mg DAB; and 20 ml of 6% hydrogen peroxide). The slide was then covered by an aqueous mounting medium (DAKO Ultramount S1964).

2.2. Morphometry and image analysis

The pictures of the Sudan stained intimal aspects of the aortas were analysed by measuring their total surface and the red stained plaque covered areas (Scion Image for Windows version Beta 4.0.2 free software). The percentage of plaque area was calculated (Fig. 1(a)).

On hematoxylin stained slides, proceeding from the proximal toward the distal end of each aorta, measurements were made at the first lipid plaque encountered and at the segment with intact intima immediately next to it (Fig. 1(b)). Measurements were performed by capturing images of media below the lipid plaques and below the normal intima. The final magnification used was 200. For medial thickness three measurements for each site were averaged (Scion Image software). In rabbits, the normal intima viewed by a light microscope consists only of a thin endothelial layer. Because of the highly variable thickness of plaques, we did not investigate the intimal plaque thickness.

The same software was used to count the number of medial smooth muscle cells. Images were density-sliced, and the nuclei were identified as dots greater than 8 pixels. Areas of medial segments were measured directly by manual highlighting. The medial smooth muscle cell density was calculated by dividing the number of nuclei by corresponding medial areas.

The peroxidase activity of the aortic media was measured on the tissue microarray slide. The technical setup included an Olympus BX-50 microscope equipped with an Olympus DP70 digital camera and corresponding software which allowed the capture of images of identical intensities. The brownish DAB reaction was quantified after white and black conversion (Adobe Photoshop 5.0 LE software) by optical densitometry, using the same threshold values throughout (Scion Image software). The mean optical densities of the groups were compared.

2.3. Statistical analysis

During evaluation of the media thickness and plaque proportion data Fisher-exact test was used (comparing control and ABAH or indometacin data and creating contingency tables). In the case of media-site results and peroxidase activity data the median-test was also applied. Smooth muscle density outcomes were also assessed by the Fisher-test. The Kruskal–Wallis ANOVA test and Mann–Whitney U post-hoc test with α-correction were applied for comparison of all media thicknesses (plaque-free and plaque-containing), controls, ABAH and indometacin data used. The level of significance was set to $p<0.05$. All results were expressed as means $\pm$ SD.

3. Results

The percent proportions of plaque sites to the whole surface area of aortas showed a tendency to increase after treatment with MPO inhibitors, however, the differences from controls were not significant. Control: $39.8\pm25.7\%$;

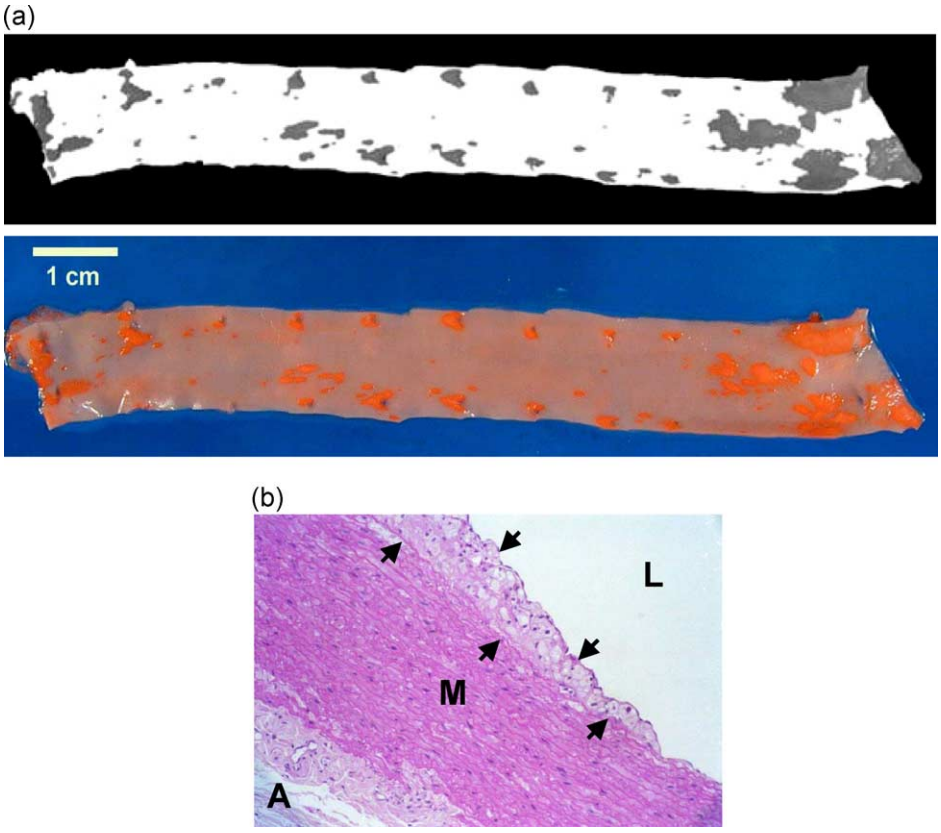

Fig. 1. (a) Intimal aspects of the thoracic and abdominal aorta stained with Sudan to highlight the lipid content of plaques. Images were converted to black and white (upper picture) The aorta was analyzed by measuring the total surface and the red stained plaque covered area, then the percentage of plaque area was calculated. (b) Aortic intimal plaque above an eosinophilic media from a cholesterol-fed (control) rabbit (L = lumen). The lower, fibrotic layer is the adventitia (A). M = media. Arrows indicate the intimal plaque. Hematoxylin–eosin 200×.

ABAH: 40.4 ± 28.6% ($p = 0.64$); indometacin: 52.5 ± 18.1% ($p = 0.24$).

Aortic media thickness of animals treated with MPO inhibitor ABAH or indometacin showed a significant increase in comparison with data of control animals in both plaque-free and plaque-containing sites of vessels. *Plaque-free area*: control group (cholesterol feeding alone): 308.4 ± 51.67 μm; cholesterol + ABAH: 375.7 ± 60.5 μm, $p < 0.05$; cholesterol + indometacin: 442.5 ± 123.4 μm, $p < 0.05$. *Plaque-containing area*: control group (cholesterol feeding alone): 349.9 ± 108.4 μm; cholesterol + ABAH: 438.5 ± 56.7 μm, $p < 0.05$; cholesterol + indometacin: 469.9 ± 122.3 μm, $p < 0.05$ (values are averages ± SD and represented in Figs. 2 and 3). Comparing all (plaque-free and plaque-containing areas) of control, ABAH and indometacin data, a marked difference was also found between control and treated groups, $p < 0.05$. No significant differences were established between the two MPO inhibitors' effect ($p = 0.328$). Although mean media thickness data from plaque-containing areas were higher in all three groups, the differences from plaque-free areas in the same study group were not significant ($p = 0.1$).

In case of smooth muscle density no significant changes were detected after treatment with MPO inhibitors. *Plaque-free area*: control group (cholesterol feeding alone): 15.3 ± 3.8; cholesterol + ABAH: 16.8 ± 3.8, $p = 0.24$; cholesterol + indometacin: 13.9 ± 1.6, $p = 1$. *Plaque-containing area*: control group (cholesterol feeding alone): 19.4 ± 7.1; cholesterol + ABAH: 17.9 ± 3.2, $p = 1$; cholesterol + indometacin: 14.6 ± 4.5, $p = 0.49$ (values are mean nuclear numbers/100 μm^2 ± SD as shown in Fig. 4).

Feeding animals with the MPO inhibitor indometacin resulted in significant decrease of peroxidase activity of plaque free vessel walls. Control: 39.8 ± 7.5, indometacin: 29.3 ± 3.8; $p < 0.05$. Although after treatment with ABAH we also found a decreasing tendency in mean values of peroxidase activity (ABAH: 34.4 ± 2.8; $p = 0.41$), it did not reach statistical significance (Figs. 5 and 6).

4. Discussion

The above findings might be considered a direct continuation of our previous studies published recently, according to which the important antioxidant effect of steroid hormones may be at least partly related to their impact on the MPO metabolism of human neutrophil leukocytes (Bekesi et al., 1997, 2000, 2001a,b). MPO may influence endothelial function, and hereby media thickness. Inhibition of MPO or of peroxidase enzyme activity of

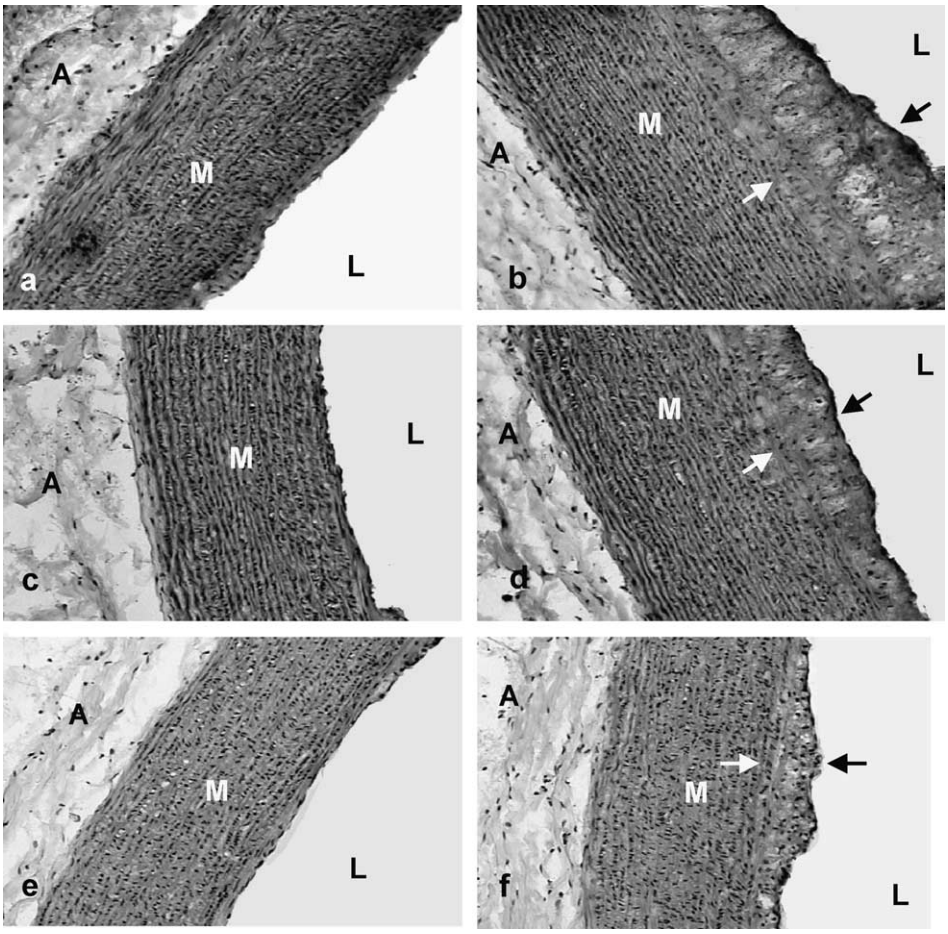

Fig. 2. Light microscopic images of aortic walls from representative animals of the different study groups. (a and b) cholesterol + indometacin, (c and d) cholesterol + ABAH and (e and f) only cholesterol-fed (control) rabbits. In (a, c, e) segments with intact, barely visible intima and in (b, d, f) adjacent segments with intimal plaques are shown. There is a medial thickening below the intimal plaques compared with the intact intima, and a thickening of the media induced by MPO inhibitors could also be detected. Hematoxylin 200×. L, lumen; M, media; A, adventitia. Arrows indicate the intimal plaque.

medial smooth muscle cells could directly induce cell proliferation. Our findings favor this hypothesis because after the administration of MPO inhibitors peroxidase activity decreased in the aortic media even in plaque free sites, parallel to which a significant thickening of the media in both plaque-free and plaque-containing areas occurred.

As treatment with MPO inhibitors increased aortic media thickness without changing the smooth muscle density, smooth muscle cell proliferation with proportional

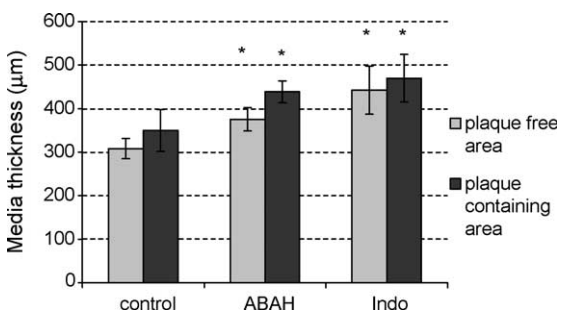

Fig. 3. Aortic media thickness of control (cholesterol-fed only) and ABAH or indometacin treated animals in plaque-free or plaque-containing areas. Data are means ± SEM. *$p < 0.05$.

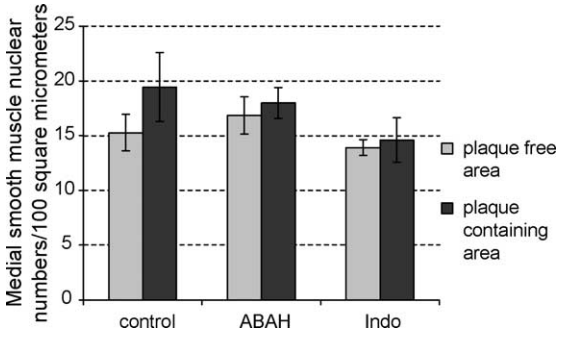

Fig. 4. Aortic media smooth muscle density of control (cholesterol-fed only) and ABAH or indometacin treated animals. Data are means ± SEM.

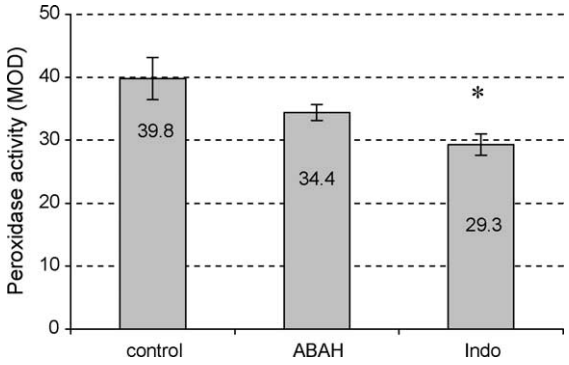

Fig. 5. Peroxidase activity of aortic vessel walls of control (cholesterol-fed only) and ABAH or indometacin treated animals. Data are averages of the mean optical densities (MOD) of the aortic media (±SEM) and reflect accumulations of peroxidase reaction products. *$p < 0.05$.

extracellular matrix accumulation might be the basis of this finding.

The results of the present work support the hypothesis that MPO or the peroxidase activity in general could have certain free radical inhibiting effects. Several contradictory data have been published in the literature regarding the role of MPO in the regulation of free radical production and its consequences in relation to human health. MPO generated oxidants have been considered to be of great importance in the pathogenesis of atherosclerosis (Wieland et al., 1993; Santanam and Parthasarathy, 1995; Podrez et al., 2000). MPO generates modified-oxidized lipoproteins via intermediate formation of hypochlorous acid (HOCL). In vitro generation of HOCL transforms lipoproteins into high uptake forms for macrophages giving rise to cholesterol-engorged foam cells. The colocalisation of immunoreactive MPO and HOCL-modified-LDL epitopes in serial sections of human atherosclerotic lesions provides further evidence for MPO–H2O2–halide system-mediated oxidation of lipoproteins under in vivo conditions. MPO could act as an important link between the development of atherosclerotic plaque in the artery wall and chronic inflammatory events (Dougherthy et al., 1994; Malle at al., 2000). According to Zhang et al. (2001) elevated levels of leukocyte and blood MPO are associated with coronary artery disease. Kutter et al. (2000) presented a protective effect of MPO deficiency against cardiovascular damage. Brennan et al. (2003) attributed a predictive role of elevated MPO levels to acute myocardial events. In their in vitro study McNally (1996) did not find any alterations in lucigenin chemiluminescence (marker of superoxide production) of MPO-deficient human neutrophils. According to several studies the MPO inhibitor indometacin seems to have a protective role against atherosclerosis (Dhawan et al., 1992; Jerlich et al., 2000).

Poliakov and Hazen found that free radical-induced peroxidation promoted by the MPO/H2O2 system of leukocytes serves as one mechanism for the generation of isolevoglandins (oxidative products of arachidonate-containing lipids and markers of oxidant stress in vivo; Poliakov et al., 2003). Kumar et al. (2004) present atherosclerosis as an alteration in which MPO is involved. Shishehbor et al. (2004) display myeloperoxidase in their work as a promising marker of cardiovascular diseases. The pseodohalide thiocyanate (high levels of it are found in plasma of smokers-risk group for cardiovascular disease) has been shown to be a suitable substrate for MPO, forming reactive oxidant, which acts as a catalyst of atherogenic modification of LDL (Exner et al., 2004). Data of Stocker et al. (2004) indicate that the principal product (hypohalous acid) of MPO imparts a defect in endothelial nitric oxide production due to a reduction in nitric oxide synthase dimer stability.

MPO has a catalyzing role in the production of several free radicals (hypochlorite anion, nitrogen dioxide, tyrosyl radicals formed with the involvement of hydrogen peroxide and made up of tyrosin, and dityrosine–the tyrosyl radical addition product). Relevant findings suggest that generation of the tyrosyl radical by means of myeloperoxidase allows activated phagocytes to damage both proteins and lipids (Heinecke, 2002). On the other hand, according to other authors MPO-dependent formation of NO-derived oxidants, and not tyrosyl radical, appears to serve as a preferred

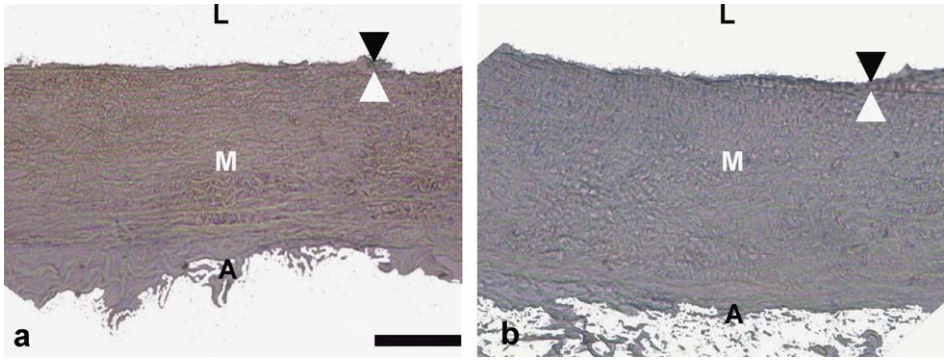

Fig. 6. Peroxidase activity reflects a difference in intensity of the brownish staining of aortic walls from a: control (cholesterol-fed only) versus b: cholesterol + indometacin-fed animals. Light microscopic image of DAB staining under the same reaction conditions (bar = 100 μm). L, lumen; M, media; A, adventitia. Arrows indicate the intimal plaque.

pathway for initiating lipid peroxidation and promoting oxidant stress in-vivo (Zhang et al, 2002). Furthermore, other results indicate that hypochlorite, and not nitrogen dioxide, generated via MPO activity, could represent one of the pathways for generation of advanced oxidation protein products in plasma proteins exposed to activated phagocytes (Capeillere-Blandin et al, 2004).

Other authors demonstrated increased oxygen consumption and augmented superoxide anion generation in a group of patients with MPO-deficiency (Badwey and Karnowsky, 1980; Klebanoff et al., 1980). The MPO inhibitor sulphite in micromolar concentrations exerted a stimulating effect on the superoxide anion production of human neutrophils in an extracellular manner (Mishara et al.,1995). Nyberg showed a stimulated lucigenin response (marker of superoxide production) by another MPO inhibitor azide (Nyberg and Klockars, 1990). According to Dima et al. (1996) superoxide is accumulated due to the MPO blockade by inhibitor azide. Further information seeming to support our working hypothesis is provided by Podrez and Hazen: modification of high density lipoprotein (HDL) with an MPO-generated tyrosyl radical system enhances the ability of this lipoprotein to support reverse cholesterol transport (Francis et al, 1993; Podrez et al, 2000; Wang et al, 1998). By enhancing transport of cholesterol to the liver from the periphery, or by oxidation of bio-molecules, tyrosyl radical may have a role both in the reduction or proliferation of vascular wall cellular elements, and thereby in media thickness.

Beyond these observations the most supportive publication in respect to our present work is the study of Brennan and colleagues (2001a), who described increased atherosclerosis in MPO-deficient mice. Furthermore, in the central nervous system of wildtype mice MPO is present in invading macrophages that produce reactive oxidants which are thought to be cytotoxic to the oligodendrocytic myelin sheath, yet contrary to expectations, the lack of MPO in MPO-KO mice significantly increased the incidence of experimental autoimmune encephalomyelitis, representing another free radical mediated disorder in which MPO may have a protective role (Brennan et al., 2001b).

Indomethacin inhibits not only MPO but the cyclooxygenase enzyme (COX), i.e. acts as a brake on synthesis of prostaglandins, known to be mitogenic in certain tissues (Nitta et al, 1991; Vane et al., 1998), and this may render its effect mechanism more complex. Despite the anti-inflammatory effect in relation to COX inhibition and the decreasing effect on production of mitogenic prostaglandins, however, in our study indomethacin also increased media thickness, similarly to ABAH. This is in conformity with the results of our earlier work (Békési et al., 2001a), where both MPO inhibitors used behaved as prooxidants. Even then we selected two enzyme inhibitors with different effect mechanisms so that we could link the results with MPO inhibition.

There are different types and species of peroxidase enzymes. Although they are similar in terms of general structure (favouring a common evolutionary origin), it is still possible to find many discrepancies among them. The literature refers to animal peroxidases as myeloperoxidase-like peroxidases. Two species belong to this family: one is myeloperoxidase, found in humans and dogs, the other is recorded in sheep and mice. Plant, fungal and bacterial peroxidases belong to the cytochrome C peroxidase-like family, which includes six further species (Dawson, 1988; Welinder, 1992). In the course of our work we used the known inhibitors of the human myeloperoxidase enzyme in rabbits. We do not have any data on the exact sub-group in which we may classify the peroxidase enzyme found in the vascular walls of these animals.

Summarizing our earlier and present data, the assumption can be made that the atherosclerotic structural aortic changes in rabbits treated with MPO inhibitors and cholesterol might be related to a decrease in peroxidase activity. The MPO and its product (hypochlorite anion) have been shown to inhibit the superoxide anion production of leukocytes, furthermore, MPO inhibitors are able to increase the free radical release from neutrophils (Békési et al., 2001a,b). Free radicals have been considered as the proximate cause of lipid peroxidation, the important initiating factor of atherogenesis. Steroid compounds which among other things (Lusis, 2000) have an impact on MPO activity might also have an antioxidant, i.e. antiatherogenic effect (Békési et al., 2001a, 2004), thus they could become appropriate for combined administration with other effective vasoprotective agents such as statins (Hodis et al., 2003).

We are well aware that animal and human data often display inconsistent tendencies. In many publications highly noteworthy of attention, MPO seems to have a prooxidant, i.e. atherogenic role. On the other hand, there are studies which are in accordance with our work hypothesis. The wide variety of methods and models used can also be responsible for the different conclusions. The picture is further complicated by the differing preponderance and frequency of cardiovascular morbidity that can be observed in respect to myeloperoxidase gene polymorphism in human studies, and by the role of estrogen in the regulation of MPO gene expression. Moreover, the MPO gene structure of individual animal species also shows differences (Kumar et al, 2004).

With regard to the contradictory viewpoints, we firmly believe on the basis of our results so far, that there is nowhere near as much antagonism in this matter as it might first appear. Pursuant to our earlier and present working hypothesis, one of the end products of the reactions catalyzed by MPO, hypochlorite anion, has a negative feedback-type inhibitive effect on the first enzyme in a chain reaction, NADPH oxidase, and thereby reduces the release of superoxide anion, an essential source of radical production in phagocytes, in similar

smooth muscle cells in the vascular wall, as well as in the endothelium (Békési et al, 2001a). We could therefore be dealing with a self-regulatory process similar to the one often seen in biological systems. The most typical example of this is the hypothalamo-hypophyseo-corticoid (thyroid, etc.) regulatory method known from endocrinology. Thus MPO produces radicals, i.e. it is prooxidant, but beyond a certain quantitative limit, counters the process of radical production by means of feedback (in biological systems we can also find other examples of a given substance having either a pro-oxidant or anti-oxidant effect, depending on its quantity; for instance Vitamin C, Feher, 1987; Cooke et al., 2003). We believe that the net outcome of these contradictory effects may depend on the prevailing unstable and sensitive equilibrium, control over which may be affected by the effect of certain steroids on MPO.

Further efforts are needed to clarify the real role of MPO in the pathogenesis of free radical mediated disorders and to answer the question, in which circumstances and respect does MPO enzyme have a harmful or a beneficial impact on living organisms. It seems necessary to elucidate how relevant animal experimental data can be interpreted under human conditions.

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References

Babior, B.M., 2000. Phagocytes and oxidative stress. Am. J. Med. 109/1, 33–44.

Badwey, J., Karnovsky, M., 1980. Active oxygen species and the functions of phagocytic leukocytes. Ann. Rev. Biochem. 49, 695–726.

Bekesi, G., Magyar, Z., Kakucs, R., Szekacs, B., Sprintz, D., Feher, J., 1997. The changes in the myeloperoxidase activity of human neutrophil granulocytes and in the amount of enzyme deriving from them under the effect of oestrogen. European Congress on Menopause, Vienna PS3-83, 51.

Békési, G., Kakucs, R., Várbíró, Sz., Rácz, K., Sprintz, D., Fehér, J., Székács, B., 2000. In vitro effects of different steroid hormones on superoxide anion production of human neutrophil granulocytes. Steroids 65/12, 889–894.

Békési, G., Kakucs, R., Sándor, J., Sárvári, E., Kocsis, I., Sprintz, D., Várbíró, Sz., Magyar, Z., Hrabák, A., Fehér, J., Székács, B., 2001a. Plasma concentration of myeloperoxidase enzyme in pre- and postclimacterial people: related superoxide anion generation. Exp. Gerontol. 37, 137–148.

Békési, G., Kakucs, R., Varbiro, Sz., Feher, J., Pazmany, T., Magyar, Z., Sprintz, D., Szekacs, B., 2001b. Induced myeloperoxidase activity and related superoxide inhibition during hormone replacement therapy. Br. J. Obstet. Gynaecol. 108, 474–481.

Békési, G., Rácz, K., Hrabák, A., Kakucs, R., Várbíró, Sz., Magyar, Z., Fehér, J., Dinya, E., Pázmány, T., Paku, S., Székács, B., 2004. Systematic investigation of different steroid precursors with respect to their effect on superoxide anion production by human neutrophil granulocytes. Horm. Metab. Res. 36 (3), 155–163.

Bots, M.L., Hoes, A.W., Hofman, A., Witteman, J.C., Grobbee, D.E., 1999. Cross -sectionally assessed carotid intima-media thickness relates to long-term risk of stroke, coronary heart disease and death as estimated by available risk functions. J. Intern. Med. 245 (3), 269–276.

Brennan, M.L., Anderson, M.M., Shih, D.M., Qu, X.D., Wang, X., Mehta, A.C., Lim, L.L., Shi, W., Hazen, S.L., Jakob, J.S., Crowley, J.R., Heinecke, J.W., Lusis, A.J., 2001a. Increased atherosclerosis in myeloperoxidase-deficient mice. J. Clin. Invest. 107, 419–430.

Brennan, M.L., Gaur, A., Pahuja, A., Lusis, A.J., Reynolds, W.F., 2001b. Mice, lacking myeloperoxidase are more susceptible to experimental autoimmune encephalomyelitis. J. Neuroimmunol. 112, 97–105.

Brennan, M.L., Penn, M.S., Lente, F.V., Nambi, V., Shishehbor, M.H., Aviles, R.J., Goormastic, M., Pepoy, M.L., Mc Erlean, E.S., Topol, E.J., Nissen, S.E., Hazen, S.L., 2003. Prognostic value of myeloperoxidase in patients with chest pain. N. Engl. J. Med. 349, 1595–1604.

Capeillere-Blandin, C., Gausson, V., Descamps-Latscha, B., Witko-Sarsat, V., 2004. Biochemical and spectrophotometric significance of advanced oxidized protein products. Biochim. Biophys. Acta 1689/2, 91–102.

Cathapermal, S., Lavigne, M.C., Leong-Son, M., Alibadi, T., Ramwell, P.W., 1998. Stereoisomerspecific inhibition of superoxide anion-induced rat aortic smooth muscle cell proliferation by 17 beta-estradiol is estrogen receptor dependent. J. Cardiovasc. Pharmacol. 31 (4), 499–505.

Cooke, M.S., Mistry, N., Ahmad, J., Waller, H., Langford, L., Bevan, R.J., Evans, M.D., Jones, G.D.D., Herbert, K.E., Griffiths, H.R., Lunec, J., 2003. Deoxycytidine glyoxal: lesion induction and evidence of repair following vitamin C supplementation in vivo. Free Radic. Biol. Med. 34 (2), 218–225.

Dai-Do, D., Espinosa, E., Liu, G., Rabelnik, T.J., Julmy, F., Yang, Z., Mahler, F., Luscher, T.F., 1996. 17 beta-estradiol inhibits proliferation of human vascular smooth muscle cells: similar effects in cells from postmenopausal females and in males. Cardiovasc. Res. 32/5, 980–985.

Das, U.N., 1992. Can free radicals induce coronary vasospasm and acute myocardial infarction?. Med. Hypotheses 39 (1), 90–94.

Dawson, J., 1988. Probing structure–function relations in heme-containing oxygenases and peroxidases. Science 240, 433.

Dhawan, V., Ganguly, N.K., Majumdar, S., Chakravarti, R.N., 1992. Effect of indomethacin on serum lipids, lipoproteins, prostaglandins and the extent and severity of atherosclerosis in rhesus monkeys. Can. J. Cardiol. 8 (3), 306–312.

Dima, V.F., Suzuki, K., Liu, Q., Koie, T., Yamada, M., Suzuki, K.J., Nakaji, S., Sugawara, K., 1996. Laser and serum opsonic activity. Roum. Arch. Microbiol. 55 (4), 277–283.

Dougherty, A., Dunn, J.L., Ratery, D.L., Heinecke, J.W., 1994. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. J. Clin. Invest. 94, 437–444.

Espinosa, E., Oemar, B.S., Luscher, T.F., 1996. 17 beta-estradiol and smooth muscle cell proliferation in aortic cells of male and female rats. Biochem. Biophys. Res. Commun. 221/1, 8–14.

Exner, M., Hermann, M., Hofbauer, R., Hartmann, B., Kapiotis, S., Gmeiner, B., 2004. Thiocyanate catalyzes myeloperoxidase-initiated lipid oxidation in LDL. Free Radic. Biol. Med. 37/2, 146–155.

Feher, J., Csomos, G., Vereckei, A., 1987. Free Radical Reactions in Medicine. Springer, Berlin.

Ferdinandy, P., Panas, D., Schulz, R., 1999. Peroxynitrite contributes to spontaneous loss of cardiac efficiency in isolated working rat hearts. Am. J. Physiol. 276:H, 1861–1867.

Francis, G.A., Mendez, A.J., Bierman, E.L., Heinecke, J.W., 1993. Oxidative tyrosylation of high density lipoprotein by peroxidase enhances cholesterol removal from cultured fibroblasts and macrophage foam cells. Proc. Natl Acad. Sci. USA 90, 6631–6635.

Heinecke, J.W., 2002. Tyrosyl radical production by myeloperoxidase: a phagocyte pathway for lipid peroxidation and dityrosine cross-linking of proteins. Toxicology 177/1, 11–22.

Heinle, H., Brehme, U., Friedemann, G., Frey, J.C., Wolf, A.T., Kelber, O., Weiser, D., Schmahl, F.W., Lang, F., Schneider, W., 2002. Intimal plaque development and oxidative stress in cholesterol-induced atherosclerosis in New Zealand rabbits. Acta Physiol. Scand. 176, 101–107.

Hodis, H.N., Mack, W.J., Azen, S.P., Lobo, R.A., Shoupe, D., Mahrer, P.R., Faxon, D.P., Cashin-Hemphill, L., Sanmarco, M.E., French, W.J., Shook, T.L., Gaarder, T.D., Mehra, A.O., Rabbani, R., Sevanian, A., Shil, A.B., Torres, M., Heiner Vogelbach, K., Selzer, R.H., 2003. Hormone therapy and the progression of coronary-artery atherosclerosis in postmenopausal women. N. Engl. J. Med. 349 (6), 535–545.

Jandl, R.C., André-Schwartz, J., Borges-Dubois, L., Kipness, R.S., Mc Murrich, B.J., Babior, B.M., 1978. Termination of the respiratory burst in human neutrophils. J. Clin. Invest. 61, 1176–1185.

Jansson, G., 1990. Oestrogen induced enhancement of myeloperoxidase activity in human polymorphonuclear leukocytes-a possible cause of oxidative stress in inflammatory cells. Free Radic. Res. Commun. 14, 195–208.

Jerlich, A., Fritz, G., Kharrazi, H., Hammel, M., Tschabuschnig, S., Glatter, O., Schauer, R.J., 2000. Comparison of HOCL traps with myeloperoxidase inhibitors in prevention of low density lipoprotein oxidation. Biochim Biophys Acta. 1481 (1), 109–118.

Joswig, M., Hach-Wunderie, V., Ziegler, R., Nawroth, P.P., 1999. Postmenopausal hormone replacement therapy and the vascular wall: mechanisms of 17 beta-estradiol's effects on vascular biology. Exp Clin Endocrinol Diabetes. 107/8, 477–487.

Kádár, A., Mózes, G., Illyés, G., Schönfeld, T., Kulka, J., Sipos, T., Glasz, T., Tőkés, A.M., Szik, Á., 1999. Histomorphometry and histochemistry of atherosclerotic lesions in coronary arteries and the aorta in young population. Wolrd Health Organization (WHO) and the World Heart Federation (WHF) Pathobiological Determinants of Atherosclerosis in Youth Study (WHO/WHF PBDAY Study) 1986-1996. Nutr. Metab. Cardivasc. Dis. 9, 220–227.

Kellogg, E.W., Fridovich, I., 1977. Liposome oxidation and erythrocyte lysis by enzimically generated superoxide and hydrogen peroxide. J. Biol. Chem. 252 (19), 6721–6728.

Kettle, A.J., Gedye, C.A., Winterbourn, C.C., 1997. Mechanism of inactivation of myeloperoxidase by 4-aminobenzoic acid hydrazide. Biochem J. 321, 503–508.

Klebanoff, S., 1980. Oxygen metabolism and the toxic properties of phagocytes. Ann Intern Med. 93, 480–489.

Kumar, A.P., Javier Piedrafita, F., Reynolds, W.F., 2004. Peroxisome proliferator-activated receptor γ ligands regulate myeloperoxidase expression in macrophages by an estrogen-dependent mechanism involving the −463GA promoter polymorphism. J. Biol. Chem. 279/9, 8300–8315.

Kutter, D., Devaquet, P., Vanderstocken, G., Paulus, J.M., Marchal, V., Gothot, A., 2000. Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit?. Acta Hematol. 104, 10–15.

Lusis, A.J., 2000. Atherosclerosis. Nature 407, 233–241.

Mack, W.J., Slater, C.C., Xiang, M., Shoupe, D., Lobo, R.A., Hodis, H.N., 2004. Elevated subclinical atherosclerosis associated with oophorectomy is related to time since menopause rather than type of menopause. Fertil. Steril. 82/2, 391–397.

Malle, E., Waeg, G., Schreiber, R., Gröne, E., Sattler, W., Gröne, H.J., 2000. Immunhistochemical evidence for the myeloperoxidase/H_2O_2/-halide system in human atherosclerotic lesions. Colocalization of myeloperoxidase and hypochlorite modified proteins. Eur. J. Biochem. 267, 4495–4503.

McNally, J.A., Bell, A.L., 1996. Myeloperoxidase-based chemiluminescence of polymorphonuclear leukocytes and monocytes. J. Biolumin. Chemilumin. 11 (2), 99–106.

Mishara, A., Dayal, N., Beck-Speier, I., 1995. Effect of sulphite on the oxidative metabolism of human neutrophils: studies with lucigenin-and luminol-dependent chemiluminescence. J. Biolumin. Chemilumin. 10 (1), 9–19.

Mohácsi, A., Kozlowszky, B., Kiss, I., Seres, I., Fülöp, T., 1996. Neutrophils obtained from obliterative atherosclerotic patients exhibit enhanced resting respiratory burst and increased degranulation in response to various stimuli. Biochim. Biophys. Acta 1316 (3), 210–216.

Mytar, B., Siedlar, M., Woloszyn, M., Ruggiero, I., Pryjma, J., Zembala, M., 1999. Induction of reactive oxygen intermediates in human monocytes by tumor cells and their role in spontaneous monocyte cytotoxicity. Br. J. Cancer 79 (5/6), 737–743.

Nitta, K., Simonson, M.S., Dunn, M.J., 1991. The regulation and role of prostaglandin biosynthesis in cultured bovine glomerular endothelial cells. J. Am. Soc. Nephrol. 2 (2), 156–163.

Nyberg, P., Klockars, M., 1990. Measurements of reactive oxygen metabolites produced by human monocyte-derived macrophages exposed to mineral dusts. Int. J. Exp. Pathol. 71 (4), 537–544.

Podrez, E.A., Abu-Sound, H.M., Hazen, S.L., 2000. Myeloperoxidase-generated oxidants and atherosclerosis. Free Radic. Biol. Med. 28 (12), 1717–1725.

Poliakov, E., Brennan, M.L., Macpherson, J., Zhang, R., Sha, W., Narina, L., Salomon, R.G., Hazen, S.L., 2003. Isolevuglandins, a novel class of isoprostenoid derivates, function as integrated sensors of oxidant stress and are generated by myeloperoxidase in vivo. FASEB J. 17/15, 2209–2220.

Salonen, J.T., Salonen, R., 1991. Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. Arterioscler. Thromb. 11 (5), 1245–1249.

San José, G., Moreno, M.U., Oliván, S., Beloqui, O., Fortuno, A., Díez, J., Zalba, G., 2004. Functional effect of the $p22^{phox}$-$930^{A/G}$ polymorphism on $p22^{phox}$ expression and NADPH oxidase activity in hypertension. Hypertension 44, 163–169.

Santanam, N., Parthasarathy, S., 1995. Paradoxical actions of anti-oxidants in the oxidation of low density lipoprotein by peroxidases. J. Clin. Invest. 95, 2594–2600.

Seligman, M.L., Mitamura, J., Shera, N., Demopoulos, H.B., 1979. Corticosteroid (methylprednisolon) modulation of photoperoxidation by ultraviolet light in liposomes. Photochem. Photobiol. 29, 549–558.

Shacter, E., Lopez, R.L., Pati, S., 1991. Inhibition of the myeloperoxidase–H_2O_2–Cl^- system of neutrophils by indomethacin and other non-steroidal anti-inflammatory drugs. Biochem. Pharmacol. 41, 975–984.

Shishehbor, M.H., Hazen, S.L., 2004. Inflammatory and oxidative markers in atherosclerosis: relationship to outcome. Curr. Atheroscler. Rep. 6/3, 243–250.

Stocker, R., Huang, AS., Jeranian, E., Hou, J.Y., Wu, T., Thomas, S.R., Keaney, J.F. Jr., 2004. Arterioscler. Thromb. Vasc. Biol. (Epub ahead of print).

Van Der Vliet, A., Smith, D., O'Neill, C.A., Kaur, H., Darley-Usmar, V., Cross, C.E., Halliwell, B., 1994. Interactions of peroxynitrite with human plasma and its constituents: oxidative damage and antioxidant depletion. Biochem. J. 303, 295–301.

Vane, J.R., Bakhle, Y.S., Botting, R.M., 1998. Cyclooxygenases 1 and 2. Annu Rev. Pharmacol. Toxicol. 38, 97–120.

Wang, W.Q., Merriam, D.L., Moses, A.S., Francis, G.A., 1998. Enhanced cholesterol efflux by tyrosyl radical-oxidized high density lipoprotein is mediated by apolipoprotein AI–AII heterodimers. J. Biol. Chem. 273, 17391–17398.

Welinder, K.G., 1992. Superfamily of plant, fungal and bacterial peroxidases. Curr. Opin. Struct. Biol. 2, 388–393.

Wieland, E., Parthasarathy, S., Steinberg, D., 1993. Peroxidase dependent metal-independent oxidation of low density lipoprotein in vitro: a model for in vivo oxidation?. Proc. Natl Acad. Sci. USA 90, 5929–5933.

Zhang, R., Brennan, M.L., Fu, X., Aviles, R.J., Pearce, G.L., Penn, M.S., Topol, E.J., Sprecher, D.L., Hazen, S.L., 2001. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 286, 2136–2142.

Zhang, R., Brennan, M.L., Shen, Z., Mac Pherson, J.C., Schmitt, D., Molenda, C.E., Hazen, S.L., 2002. Myeloperoxidase functions as a major enzymatic catalyst for initiation of lipid peroxidation at sites of inflammation. J. Biol. Chem. 277/48, 46116–46122.

TOVÁBBKÉPZŐ KÖZLEMÉNYEK

Gyógyszerészet 46. 67–71. 2002.

Intellingens polimerek alkalmazása a gyógyszer-technológiában

I. Általános fogalmak, lehetőségek

Dinya Mariann és dr. Antal István

Bevezetés

Az anyagtudomány fejlődésével, a kémiai szerkezet és tulajdonságok közötti kapcsolatok felismerésével lehetővé vált adott célra tudatosan, mérnöki módon tervezett anyagok (engineered materials) előállítása, amelyek különböző előnyös – pl. mechanikai, elektromos, termikus – tulajdonságokkal rendelkeznek [1]. Az anyagok egy régebben ismert csoportja a mindennapi környezeti feltételek mellett passzívan érintkezik környezetével, és ez a változatlanság a hosszú élettartam feltétele. Ugyanakkor az aktív anyagok környezeti behatásra tulajdonságaik (pl. méret, alak vagy fényáteresztőképesség, viszkozitás, vezetőképesség stb.) megváltoztatásával válaszolnak ***(1. ábra),*** ami funkcionálisan kihasználható.

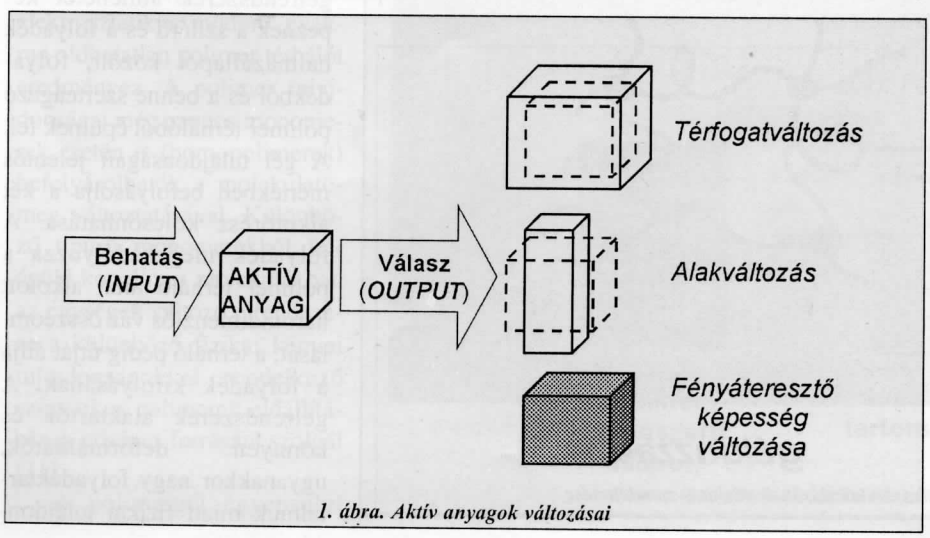
Néhány elem esetében az elektromos tulajdonság fényhatással hozható összefüggésbe (fotoelektromosság), például a szelén vezetőképessége megnő megvilágítás hatására, vagy a szilícium-napelem a fényerősség változását feszültséggé alakítja át. *Pierre és Paul Jacques Curie* 1880-ban fedezték fel a piezoelektromos hatást, amikor a kvarckristályban (SiO_2) mechanikai igénybevétel (pl. nyomás) hatására elektromos áram keletkezhet. Deformálódás következtében ugyanis kisebb és nagyobb potenciálú gócok alakulnak ki a kristályban illetve annak felületén, megindítva az elektronok vándorlását. A folyamat reverzibilis, elektromos áram hatására a kvarckristály mechanikai rezgéseket végez (napjainkban pl. a kvarcórában).

Az anyag tulajdonságbeli változása utal az azt előidéző igénybevételre, ezért lehetőséget adhat érzékelő funkció ellátására (szenzor). Fordított esetben, a tulajdonságbeli változást kísérő alakváltozást kihasználva, az anyag mozgást elindító (aktuátor) szerkezetben alkalmazható.

Az USA-ban 1967-ben fejlesztették ki a fototrop üveget [2], amely fénytől függő pigmentálódását a szubmikroszkópikus nagyságú és mintegy 1:2000 arányú ezüst-halogenid kristályoknak köszönheti. A fototrop szemüvegek fényáteresztő képessége alkalmazkodik a fényviszonyokhoz, az elsötétedés oka, hogy fény hatására fémezüst válik ki. A gyakorlati alkalmazhatóságot a különleges technikai megoldás biztosítja, vagyis a reakció visszafordíthatósága, bár az újra kivilágosodás lassabban megy végbe.

Az anyagtudomány egyik feladata az ilyen alkalmazkodóképességgel rendelkező aktív anyagok kutatása. Az említett jelenségek az anyag és közvetlen környezete közötti mechanikai, kémiai, termodinamikai egyensúlyi állapottal magyarázhatók. A változó fizikai vagy kémiai körülményeknek megfelelően új egyensúlyi állapot alakul ki, amelyben az anyag már más tulajdonságokkal rendelkezik. A környezeti hatás viszonylag kis különbségeire az anyag jellemző és nagymértékű tulajdonságbeli változással válaszol.

Az „intelligens" viselkedés az ilyen típusú anyagok sajátsága, így ezek különböznek a mérnöki technika révén előállított intelligens rendszerektől illetve szerkezetektől (www.intellimat.com/materials/overview/: materials, devices, systems, structures). Tekintettel az alkalmazkodóképességre és az érzékelő funkcióval összefüggő „felfogóképességre", valamint a folyamatra jellemző inger-válasz kapcsolatra, a csoporthoz tartozó anyagokra használják többek között az „intelligens anyagok" (intelligent materials), „furfangos anyagok" (smart materials), „alkalmazkodó anyagok" (adaptive materials) vagy egyszerűen „aktív anyagok" (active materials) elnevezéseket. (Úgy tűnik, hogy fejlődése során a technika kénytelen az élettudományoktól kölcsönözni kifejezéseket, pl. az elmúlt évti-



1. ábra. Aktív anyagok változásai

zedben már megszoktuk az ártalmas és fertőző számítógépes programokra használt „vírus" elnevezést.)

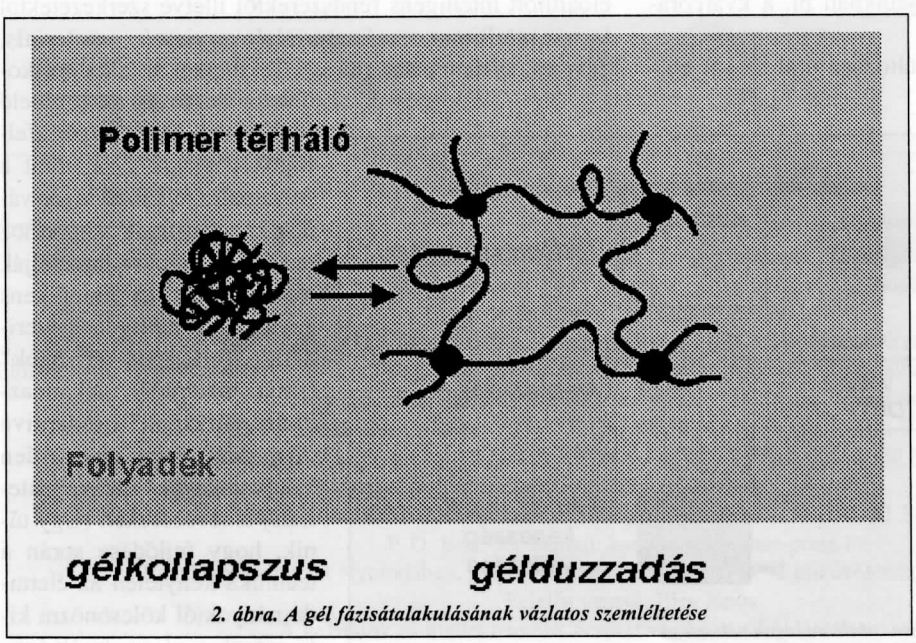
Az intelligens anyagok egy jelentős hányadát alkotják azok a szilárd anyagok, amelyeknek mérete illetve alakja elektromos, mágneses, vagy hőmérsékleti hatásra változik meg (elektro-, magneto-, termostrikciós jelenség). Bizonyos fémötvözeteknél (pl. réz-cink-alumínium) a méretváltozás olyan kicsi, hogy ezekből az anyagokból nagypontosságú pozicionáló eszközök vagy pl. tűzvédelmi berendezések készíthetők [3]. Az ún. emlékező fémeket és műanyagokat az élet igen sok területén alkalmazzák és felhasználási körük egyre bővül, különösen az orvostudomány és a robottechnika területein. Legismertebb ilyen anyag az USA-ban 1958-ban felfedezett Nitinol, amely a fémötvözet alkotórészeinek (nikkel és titán) kezdőbetűiből és az anyagot előállító laboratórium (Naval Ordnance Laboratory) kezdőbetűiből kapta nevét. Az alakmemória megőrzésének alapja jellegzetes kristályszerkezeti átalakulás (martenzit-szerkezet), amely hűtéssel és/vagy mechanikai feszültség alkalmazásával is kialakítható. A hőmérséklet csökkentése mellett alkalmazott mechanikai feszültség irányítottságot visz a kristályszerkezetbe. Az új fázisban szimmetrikus ikerkristályokból álló, egymással párhuzamos sávok képződnek, amelyek az eredő feszültség irányában rendeződve „befagyasztják" az anyag belsejében a feszültségeloszlási mintát. Az anyag eredeti, kritikus hőmérséklet felett létrehozott alakját az irányított kristályszerkezet révén lehűtve is megőrzi, majd melegítés hatására visszanyeri kezdeti formáját. Megfelelő ötvözéssel a kritikus hőmérséklet az emberi test hőmérsékletére is beállítható, ezért az emlékező anyagokat eredményesen használhatják az orvosi gyakorlatban értágító- vagy csontműtéteknél. Mivel ezek az anyagok jelentős erőt képesek kifejteni miközben visszanyerik eredeti alakjukat, ezért robotok készítésénél is alkalmazást nyerhetnek [4].

A mechanikai behatásokkal szemben kismértékű tehetetlenséggel rendelkező lágy anyagok (soft materials) csoportja folyékony vagy képlékeny halmazállapotú. Folyadékok is rendelkezhetnek intelligens tulajdonságokkal, mint például a magneto- és elektro-reológiai folyadékok. Ezeknél a reológia jellemzők (pl. viszkozitás és folyáshatár) a mágneses illetve elektromos tér nagyságától függenek, alkalmazásuk elsősorban erőátviteli rendszerekben várható [5, 6]. A már forgalomba került viszkoelasztikus tulajdonságú SmartGel szobahőmérsékleten lágy és hajlítható, testhőmérsékleten viszont keményebb lesz, így cipőbetétként használják, kényelmesebbé téve a viseletet [7].

Mivel a folyadékokon és rugalmas műanyagokon kívül ebbe a csoportba tartozik a biológiai anyagok nagy többsége, így a lágy anyagok intelligens tulajdonságának kutatása több előnyös felhasználási lehetőséget teremt az orvostudomány mellett a gyógyszertudományok területein is, különösen a gyógyszer-technológiában.

Intelligens makromolekulás gélek

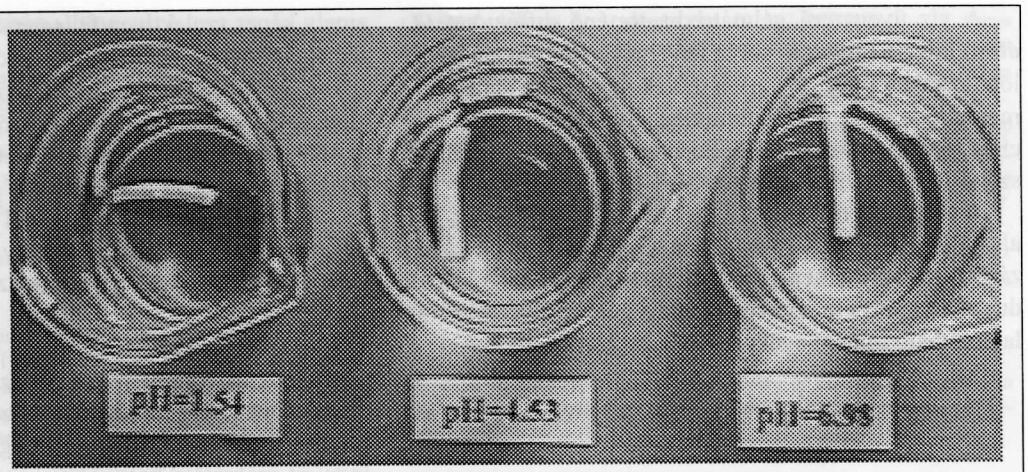
1975-ben *Tanaka* poliakrilgélek tanulmányozása közben arra a jelenségre lett figyelmes, hogy a vizsgált gél hűtve (–20 °C) opálos lesz, majd felmelegítve visszanyeri tisztaságát [8]. A gélrendszerben tapasztalható jelenséget olyan fázisátalakulással magyarázta, amely hasonló a víznél ismert folyadék-gőz átmenethez. A fázisátalakulást szobahőmérsékleten is megvalósítva kizárta az opaleszcenciát adó jégkristályok keletkezését, így sikerült meggyőznie a magyarázatban kételkedőket. *Tanaka* megfigyelte, hogy az előállított gélek az élő szervezetekhez hasonlóan válaszolnak a külső környezeti ingerekre, felfedezéseivel új kutatási területet nyitott meg. A biológiai vonatkozások miatt legígéretesebb és ezért legtöbbet tanulmányozott anyagok a hidrogélek, melyek a környezeti hatások egészen kis változására is élesen reagálnak, pl. 1 °C-os hőmérséklet változtatás térfogatuk sokszoros növekedéséhez vagy éppen csökkenéséhez vezethet. Az utóbbi esetben folyadéktartalmuk 90%-át is elveszíthetik.



2. ábra. A gél fázisátalakulásának vázlatos szemléltetése

A kolloid koherens rendszerek közé tartozó makromolekulás gélrendszerek átmenetet képeznek a szilárd és a folyadék halmazállapot között, folyadékból és a benne szerteágazó polimer térhálóból épülnek fel. A gél tulajdonságait jelentős mértékben befolyásolja a két alkotórész kölcsönhatása. A folyadék megakadályozza a polimer térháló által alkotott háromdimenziós váz összeomlását, a térháló pedig útját állja a folyadék kifolyásának. A gélrendszerek alaktartók és könnyen deformálhatók, ugyanakkor nagy folyadéktartalmuk miatt fizikai tulajdon-

ságaik az oldatokéhoz hasonlóak. A gél konzisztenciája a kémiai összetételtől és más tényezőktől függően a viszkózus folyadéktól a szinte merev szilárd állapotig változhat, de általában lágy és rugalmas, vagyis a legegyszerűbb kifejezéssel élve „zselés".



3. ábra. Hidrogél duzzadása optimális pH-értéken [21]

A gélnek a környezeti feltételekre adott különböző válaszai a kritikus jelenségek illetve fázisátalakulások alapján értelmezhetőek. Míg számos anyag folyékony vagy gáz állapotban fordul elő különböző körülmények között, addig a gélek a polimer térháló konfigurációja által megkülönböztethetően kétféle fázisban létezhetnek. A forrásban lévő vízhez hasonlóan a gél térfogatának és egyéb tulajdonságainak nemfolytonos változása a fázisok közötti hirtelen átmenettel jellemezhető. A kritikus pontban a polimer térháló tulajdonságai (pl. sűrűsége, pórusmérete stb.) nagymértékben ingadoznak. Az állapotváltozások alapjául szolgáló folyamatok jellemzőek a gél szerkezetére és csak a polimer térhálóban ható erők tanulmányozásával válnak értelmezhetővé.

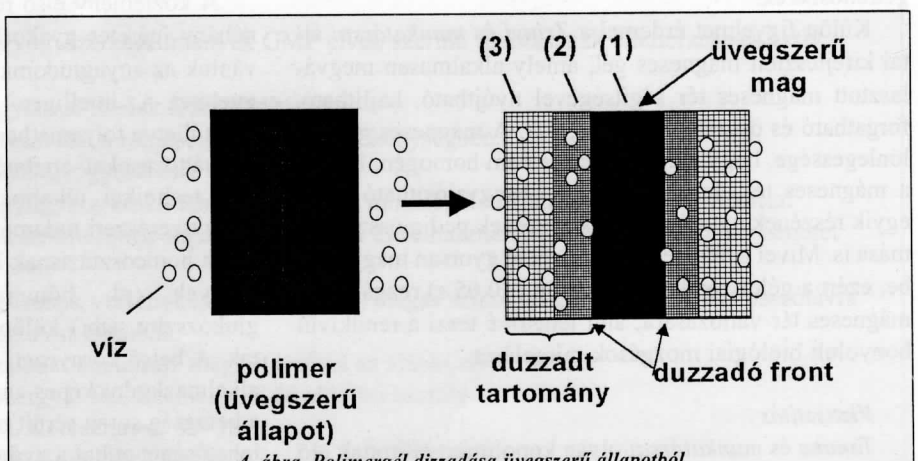
A vázként szolgáló makromolekulák közötti kölcsönhatások négy típusba sorolhatók [9]: ionos, hidrofób, van der Waals és hidrogénhíd kötések. A folyadékfázis szolvatáció révén, kapilláris erőkkel vagy mechanikailag bezárva kapcsolódhat a gélvázhoz [10].

A különböző polimerek egyedi méretbeli és háromdimenziós szerkezetbeli tulajdonságokkal rendelkeznek. Kémiai reaktivitásukat elsősorban a monomeregységek kémiai jellemzői határozzák meg, de tulajdonságaik nagymértékben függnek a monomerek kapcsolódásától is. A polimer lánc alapvetően egyenes vagy elágazó lehet, ugyanakkor a láncok keresztirányú összekapcsolása (kovalens vagy elektrosztatikus módon) gyakran oldhatatlan polimer térhálót eredményez. A polimer tulajdonságai még azonos monomerek esetén is (homopolimerek) befolyásolhatók a molekulatömeg változtatásával. Különböző típusú monomerekből felépülő kopolimer molekulákban az egységek változó elrendezése a különböző fizikai kémiai tulajdonságokkal rendelkező szintetikus polimerek előállításának bőséges forrásául szolgál [11].

A polimergél egyensúlyi állapotban lehet környezetével, ilyenkor a jellemző egyensúlyi térfogat a folyadékmolekulák duzzasztó (ozmotikus) hatásának, valamint a polimer molekulákban a deformáció ellen ébredő visszahúzó erő hatásának a következménye. A polimer térháló tágulása általában elektrosztatikus jellegű taszító intermolekuláris erőknek tulajdonítható [9].

A környezeti paraméterek módosulására a gél térfogatának változtatásával válaszol ***(2. ábra)***. E térfogatváltozás lehet folytonos vagy pl. ugrásszerű zsugorodás (gélkollapszus), amely a gél szerkezetétől függő kritikus hőmérsékleten játszódik le. A gél térfogatváltozása a hőmérsékleten [12] kívül előidézhető a pH [13], ion- és elegyösszetétel [14], fényintenzitás[15] megváltoztatásával valamint elektromos [16] illetve mágneses [17] tér alkalmazásával.

Tekintettel arra, hogy sokféle környezeti hatásra képesek reagálni, a polimergélek különleges helyet foglalnak el az intelligens anyagok között. A gélkollapszus, vagy ennek ellentéte, a nagymérvű duzzadás, az említett környezeti paraméterek kritikus pont körüli kismérvű változtatásával érhető el. A gél térfogatmódosulása igen jellemző ***(3. ábra)***, de azt más változások is kísérhetik, így pl. az optikai (transzparencia-opaleszcencia), mechanikai illetve az oldott anyag diffúzióját meghatározó transzporttulajdonságok is módosulhatnak [18–20].



4. ábra. Polimergél dizzadása üvegszerű állapotból

A víz üvegszerű gélmátrixba történő diffúzióját ***(4. ábra)*** követő duzzadás során lényegében három lépés különböztethető meg: (1) a víz molekulák diffúziója a polimer térháló közé, (2) a polimerlánc hidratációja és relaxációja, (3) a polimerlánc fellazulását követően a térháló szétterjedése a vizes közegbe [21].

A duzzadás folyamata egyszerűen jellemezhető a gél alakjának (pl. gömb, korong, henger stb.) méretbeli változásával, illetve a számított térfogati értékekkel [22]. A duzzadás aránya tanulmányozható a gél vízfelvételnek tulajdonítható tömegnövekedéséből is [23]:

$$DI_m = \frac{m_t - m_0}{m_0} \qquad (1)$$

ahol DI_m a duzzadási index, m_0 és m_t a gél tömege kezdetben, illetve t idő után.

Intelligens gélek alkalmazásának néhány ígéretes lehetősége

A környezeti ingerre válaszoló polimergélek előállítása és tulajdonságaiknak tanulmányozása gyorsan fejlődő kutatási területté vált. A lágy robottechnika, a biológiai rendszerekhez hasonló, azokat utánzó energiaátalakító rendszerek, mikroszerkezetek, szelektív szorbensek, szenzorok és a szabályozott hatóanyagleadó rendszerek fejlesztése képezik a kutatások fő irányait.

Mesterséges izom

A gél térfogatváltozása terhelés során is bekövetkezik, ha a duzzadó gélt terhelésnek vetjük alá, azaz felszínére súlyt helyezünk, vagy az összehúzódó géllel tömeget mozdítunk el [24]. A térfogatváltozás alkalmas mechanikai munkavégzésre, valamint különleges alakváltozások és mozgások megvalósítására.

Ha a környezeti hatás kémiai természetű, akkor az energia hasznosításának az izomra jellemző módja valósul meg. Ez a különleges tulajdonság már a negyvenes évek végétől inspirálta a kutatókat mesterséges izmok és új típusú gépek kifejlesztésére [25]. A mozgást kiváltó hatás alapján megkülönböztethetők termikus, elektrosztatikus, kémiai, elektrokémiai és mágneses hatással aktivált gélrendszerek.

Külön figyelmet érdemel a *Zrínyi* és *munkatársai* által kifejlesztett mágneses gél, amely alkalmasan megválasztott mágneses tér segítségével nyújtható, hajlítható, forgatható és összehúzható [26, 27]. A mágneses gél különlegessége, hogy a deformációja nem homogén, hanem a mágneses tér eloszlásától függ. Megvalósítható a gél egyik részének nyújtása, másik részének pedig összenyomása is. Mivel a mágneses polarizáció gyorsan megy végbe, ezért a gélek rendkívül gyorsan (<0,05 s) reagálnak a mágneses tér változására, ami lehetővé teszi a rendkívül bonyolult biológiai mozgások mímelését.

Víztisztítás

Tanaka és *munkatársai* olyan kopolimert állítottak elő poliakrilsav és poli-N-izopropilakrilamid segítségével, amely képes szelektíven felismerni és megkötni a nehézfémeket [28]. 37 °C-on a polimer duzzadt állapotban van, így a kelátképző csoportok távol vannak egymástól. Ugyanakkor a gél melegítve (kb. 50 °C) zsugorodni kezd és ebben az állapotában magába tudja zárni a fémet. A gél fázisátalakulásának hőmérséklete minden fémnél más és csökkentve a hőmérsékletet, a gél térfogatnövekedésével a fémion ismét felszabadul. Az elv új technológia bevezetését teszi lehetővé a víztisztítás számára.

Mesterséges pancreas

Kísérletes eredmények [29] azt mutatják, hogy mesterséges pancreas állítható elő hőérzékeny poli-N-izopropil-akrilamid és poliakrilsav együttes alkalmazásával. Inzulint szekretáló hasnyálmirigy sejteket tartalmazó folyékony polimer oldat féligáteresztő membránba csomagolva a testbe implantálható. 37 °C-on az oldatból gél lesz, ami rögzíti a sejteket, s azok a vér glukózszintjének megfelelően szekretálni képesek az inzulint. Bár a beültetett Langerhans-sejtek maximum egy évig életképesek, de a csere könnyen megoldható: jegelni kell a testrészt, mire a gél elfolyósodik.

SmartHydrogel alkalmazása szemcseppekben

A SmartHydrogel polimer összetevői a bioadhezív és pH-érzékeny poliakrilsav (PAA) és egy polipropilén-oxidot (PPO) és polietilén-oxidot (PEO) tartalmazó kopolimer (Pluronic). Míg a hagyományos szemcseppeket a könny hamar felhígítja és kimossa, a SmartHydrogel a szembe cseppentve még folyékony, de testhőmérsékleten viszkózussá válva megnyújthatja a gyógyszerek hatástartamát. Ugyanakkor a gél nyíróerőkre érzékeny, minden pislogásnál pillanatszerűen elfolyósodik. Ez biztosítja a hatóanyag fokozatos felszabadulását és egyenletes eloszlását. Testhőmérsékleten a hidrofób PPO részek micellát képezve aggregálódnak, ezáltal lehetőséget teremtenek vizes közegben a lipofil hatóanyagok szolubilizálására és felszabadítására is [30].

Összefoglalás

A közlemény első részében az általános fogalmak és néhány ígéretes gyakorlati alkalmazás áttekintésével kívántuk az anyagtudomány egy új területére.felhívni a figyelmet. Az intelligens anyagok kutatása a már megszületett illetve folyamatban lévő fejlesztések révén forradalmi változásokat eredményezhet nemcsak a mindennapi élet technikai alkalmazásaiban, hanem az orvosi és gyógyszerészeti tudományok területein is. Szervezetünkben a homeosztázisnak köszönhetően a fiziológiás körülmények (pl. hőmérséklet, pH, ionkoncentráció, glukózszint, stb.) különleges pontossággal szabályozottak. A belső környezeti feltételeket érzékelni és azokhoz alkalmazkodni képes anyagok alkalmazása hozzájárulhat a betegség során sérült működés helyreállításához, illetve lehetőséget adhat a gyógyszeres terápia hatékonyságának optimálásához.

IRODALOM

1. *Zrínyi M.:* Intelligens anyagok. Magyar Tudomány *6,* 697–703 (1999). – 2. *Paturi, F. R.:* Chronik der Technik. Bodo Harenberg und Chronik Verlag, Dortmund, 1988. – 3. *Juhász A. Tasnádi P.:* Érdekes anyagok, anyagi érdekességek. Akadémiai Kiadó, Budapest, 1992. – 4. *Kauffman, G. B.; Mayo, I.:* The Thermobile™: A Nitinol-Based Scientific Toy. J. Chem. Educ. *75,* 313 (1998). – 5. *Garrido, L.:* Complex Fluids. Springer Verlag, 1993. – 6. *Fendler, J., H., Dékány, I.:* Nanoparticles in Solids and Solutions. Kluwer Academic Press, 1997. – 7. *Dagani, R.:* Intelligent Chem. Eng. News, 75, 1997. június – 8. *Tanaka, T.:* „Gels," Sci. Amer. *244,* 124 (1981). – 9. *Ilmain, F., Tanaka T., Kokufuta E.:* „Volume Transition in a Gel Driven by Hydrogen Bonding." Nature *349,* 400–401 (1991). – 10. *Rácz I., Selmeczi B.:* Gyógyszer-technológia III. Medicina Könyvkiadó, Budapest, 2001. – 11. *Florence, A., T., Atwood, D.:* Physicochemical principles of pharmacy. 3rd Ed.,Macmillan Press, London, 1998. p. 309–311. – 12. *Hirotsu, S., Hirokawa, Y. and Tanaka, T.:* J. Chem. Phys. *87,* 1392–1395 (1987). – 13. *Kuhn, W., et. al.:* Nature *165,* 514–516 (1950). – 14. *Ohmine, I. and Tanaka, T.:* J. Chem. Phys. *77,* 5725 (1982). – 15. *Suzuki, A., Tanaka, T.:* Nature *346,* 345–347 (1990). – 16. *Tanaka, T., Nishio, I., Sun, S. T., Ueno-Nishio, S.:* Science *218,* 467 (1982). – 17. *Annaka, M., Tanaka, T.:* Nature *355,* 430–432 (1992). – 18. *Okano, T.:* Biorelated Polymers and Gels. Academic Press, San Diego, 1998. – 19. *Okano, T.:* Intelligent Materials *9,* (3), 3–5 (2000). – 20. *Yoshida, R., Okuyama, Y., Sakai, K., Okano, T., Sakurai, Y.:* J. Membr. Sci. *89,* 267–277 (1994). – 21. *Dinya M.:* Környezeti hatásokra reagáló hidrogélek szerepe a szabályozott hatóanyagleadásban. XXV. OTDK, Pécs, 2001. április 4–7., Absztrakt p. 40. – 22. *Li, Y., Tanaka, T.:* J. Chem. Phys. *92,* 1365–1371 (1990). – 23. *Andreopoulos, A.G.,Tarantili, P. A.:* J. Biomat. Appl. *16,* 34–46 (2001). – 24. *Zrínyi, M.:* Intelligens anyagok. Magyar Tudomány *6,* 682–688 (1999). – 25. *Kuhn, W.:* Experientia *5,* 318 (1948). – 26. *Zrínyi M., Gács J., Simon Cs-né:* 213 215 sz. Magyar Szabadalom. – 27. *Zrínyi, M.:* Trends in Polymer Sci. *9,* 277 (1997). – 28. *Tanak, T. et al.:* Faraday Discuss. *101,* 201 (1995) – 29. *Jeong, B.M., Bae, Y. H., Lee, D. S., Kim, S. W.:* Nature *388,* 860 (1997). – 30. 5,597,810 sz. USA szabadalom.

M. Dinya and I. Antal: ***Application of intelligent polymers in pharmaceutical technology. Part I. General considerations and perspectives***

The review focuses on intelligent polymer gels (hydrogels) which may respond sharply to various external stimuli (temperature, pH, ionic strength, light, electric and magnetic field etc.) exhibiting large changes in volume, optical transmission, surface energy and permeability for mass transfer process. The potential application of these polymers for drug delivery is under intensive investigation in order to optimize the effectiveness of drug therapy.

Semmelweis Egyetem, Gyógyszerészeti Intézet, Budapest, Hőgyes Endre u. 7. – 1092

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Intellingens polimerek alkalmazása a gyógyszer-technológiában

II. A hatóanyagleadás szabályozására alkalmas mechanizmusok

Dr. Antal István és Dinya Mariann

Bevezetés

A hatóanyagleadás szabályozásának célja a hatóanyag jellemző sebességgel történő eljuttatása meghatározott helyre, annak érdekében, hogy adott időben megfelelő mennyiségben legyen jelen a hatás helyén.

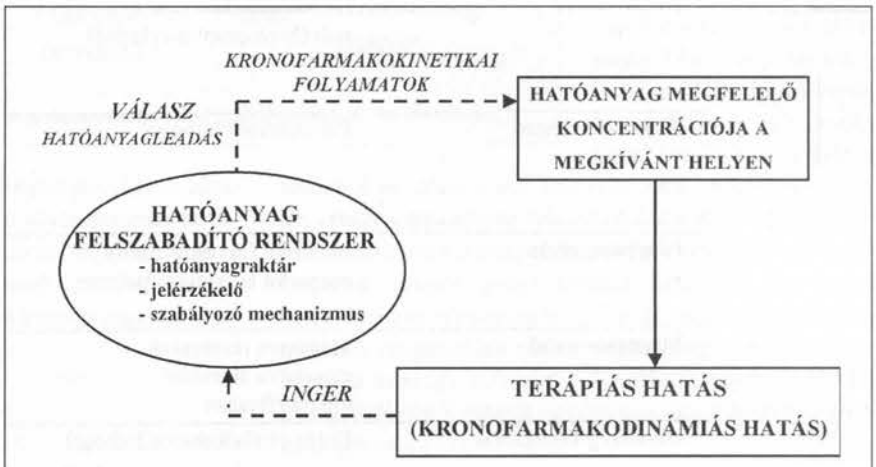
A szabályozott hatóanyagleadás biztosítására alapvetően a hatóanyagot raktárban (rezervoár) vagy monolitikus (mátrix) rendszerben tartalmazó megoldások szolgálhatnak [1–5]. Előbbi esetben a hatóanyag elsősorban diffúzió, utóbbi esetben erózió révén szabadul fel. A folyamatos hatóanyagfelszabadulás szabályozásának elvi alapját képezheti [6]:

– a hatóanyag passzív diffúziójának sebességét befolyásoló polimerbevonat alkalmazása membránbarrierként,
– oldódás-kontrollált felszabadulás,
– ozmotikus pumpa vagy
– duzzadás által szabályozott felszabadulás.

Hosszabb időn át a nulladrendű hatóanyagleadási kinetikát elérő technológiai megoldások (pl. mikrokapszulák, vázszerkezetek, ozmotikus rendszerek) kerültek előtérbe, amelyekkel biztosíthatóvá vált a hatóanyag folyamatosan, állandó sebességgel történő szervezetbe juttatása. E célkitűzés megvalósítása az adagok számának csökkentése révén lehetővé tette a beteg életminőségének javítását, miközben az állandó hatóanyagkoncentráció megnövelte a terápiás hatékonyságot illetve a vérszint ingadozásainak minimalizálásával mérsékelte a mellékhatások kialakulását.

Figyelembevéve a szervezet biológiai ciklusait, ma már ismert, hogy számos betegség a kronofarmakokinetika és kronofarmakodinámia alapján kezelhető leghatékonyabban [7]. A fiziológiás paraméterek ingadozása következtében a megnövelt terápiás hatóanyagkoncentráció iránti igény egy adott napszakban jelentkezik számos betegségnél (asthma, szív és keringési betegségek, arthritis stb.). Ebben az esetben előnyösebb, ha a hatóanyag felszabadulása visszacsatolásos (biofeedback) elv alapján szakaszosan, az aktuális fiziológiás igények szerint megy végbe. A szakaszos hatóanyagleadás eredményeként kialakuló hatóanyagkoncentráció oszcillál, így alkalmas lehet a fiziológiás körülmények utánzására (*mimicking*). Az oszcilláló, vagy más néven pulzáló hatóanyagleadás iránti igény különösen a hormonterápiában mutatkozik meg [8, 9].

Az ***1. ábra*** ingerérzékeny (*stimuli-sensitive*), a fiziológiás környezeti ingerre a hatóanyag leadásával válaszoló rendszer működési elvét vázolja. Ilyen típusú hatóanyagfelszabadító rendszer megvalósítására ad lehetőséget intelligens makromolekulás gélek gyógyszer-technológiai felhasználása. A polimergél alkalmas a benne eloszlatott hatóanyag raktározására és a környezeti feltételektől függő tulajdonságbeli változás egyben jelérzékelőként illetve szabályozó mechanizmusként szolgál. A gélállapot módosulását az oldott anyag diffúzióját illetve a hatóanyagleadás képességét meghatározó transzporttulajdonságok változása kíséri.



1. ábra. Inger-érzékeny hatóanyag-felszabadító rendszer működési elve

Kronofarmakokinetika: az a szakterület, amely a hatóanyagnak a felszívódását, megoszlását, metabolizmusát, eliminációját jellemző farmakokinetikai paraméterek változásait tanulmányozza a fiziológiás körülmények (pl. vérnyomás, hőmérséklet stb.) időszakos (pl. napszaki) ingadozásaival összefüggésben.

Kronofarmakodinámia: az a szakterület, amely a gyógyszerhatással összefüggő fiziológiás és biokémiai változásokat az élettani folyamatok időszakos (pl. napszaki) ingadozásaival összefüggésben kutatja.

A hatóanyagleadás szabályozásának elvi alapjai

Tekintettel arra, hogy gélekben a hatóanyag oldódását és diffúzióját a vízfelvétel illetve a polimer térháló kiterjedése határozza meg, a környezeti feltételektől befolyásolt duzzadás vagy zsugorodás nyilvánvaló megoldást kínál a hatóanyag felszabadulásának szabályozására. Hidrogél rendszerben a hatóanyag relatív mobilitása a határfelületi duzzadási számmal (*swelling interface number,* S_w) jellemezhető:

$$S_W = \frac{v\delta(t)}{D} \qquad (1)$$

ahol v a penetráló folyadék által eredményezett mozgó front sebessége, $\delta(t)$ a hatóanyag diffúzióját biztosító duzzadt réteg vastagsága és D a hatóanyag diffúziós koefficiense a duzzadt rétegben [10].

A hatóanyagleadás kinetikája polimergélből általában az alábbi egyenlettel modellezhető [11]:

$$\frac{M_t}{M_\infty} = kt^n \qquad (2)$$

ahol M_t a t időben felszabadult hatóanyagmennyiség, M_∞ a végtelen időben felszabaduló hatóanyag mennyisége, k a polimer rendszer szerkezeti és geometria tulajdonságait magában foglaló sebességi állandó, n a diffúzió transzport mechanizmusát jellemző hatványkitevő. Duzzadás által szabályozott hatóanyagfelszabadulás esetén alapvetően fontos a hatóanyagleadás és a gél viselkedése közötti összefüggések tanulmányozása ***(I. táblázat).***

I. táblázat

A hatóanyagleadás kinetikai modellje duzzadás által szabályozott hatóanyagfelszabadulás esetén

Transzport mechanizmus	$M_t/M_\infty = kt^n$	S_w
Fick-féle diffúzió	n = 0,5	>>1
Anomáliás transzport	n > 0,5	≈1
„Időfüggetlen" (0.-rendű kinetika)	n = 1	<<1

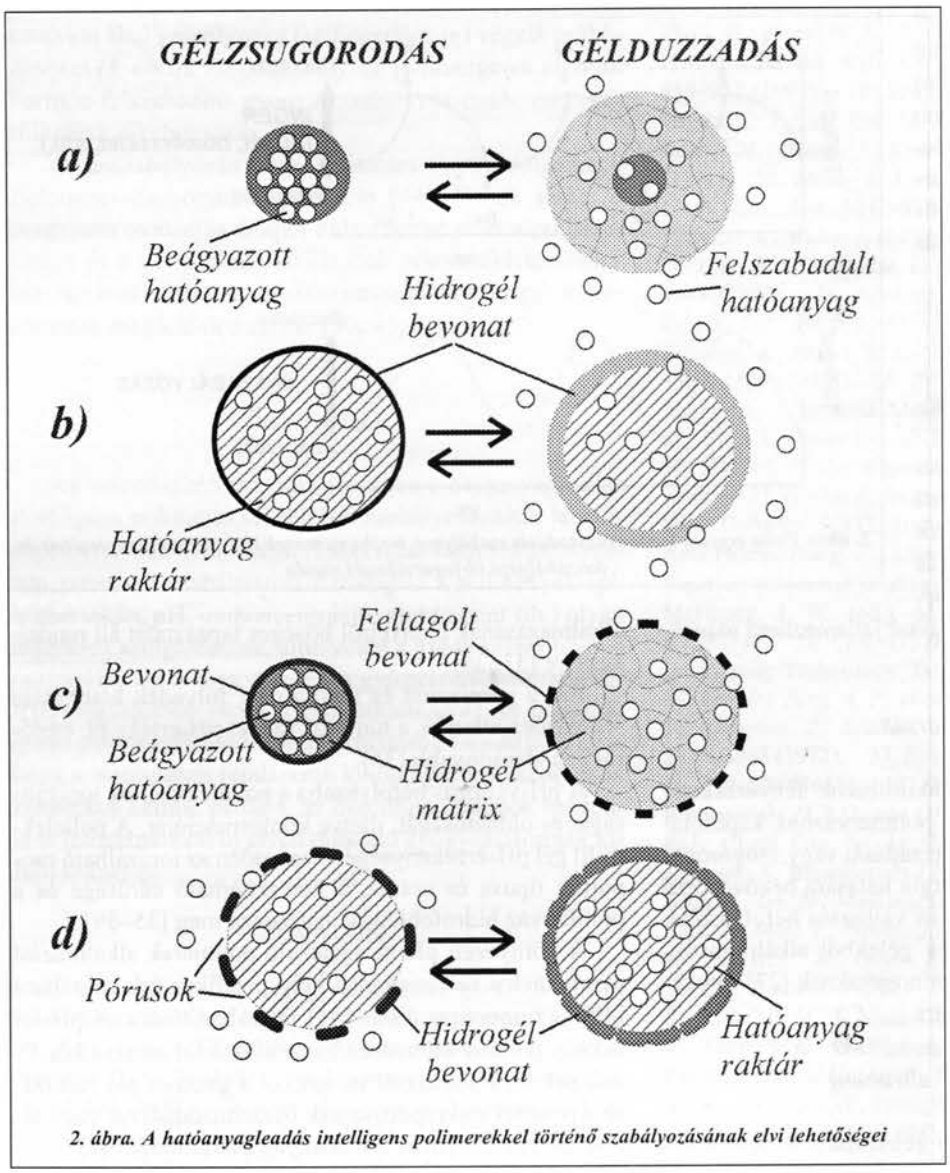
Szabályozó mechanizmusok

A környezeti paraméterek módosulására a gél térfogatának változtatásával válaszol, a duzzadás vagy zsugorodás különbséget eredményez a hatóanyagleadásban. Néhány ilyen elvi lehetőséget a ***2. ábrán*** vázolt esetek (*a-d*) szemléltetnek. Egyszerű megoldást jelenthet a hatóanyag gélmátrixba ágyazása (*a*), amikor a hatóanyag diffúzióját a gél duzzadása teszi lehetővé. Hasonló elv érvényesíthető úgy is, ha gélmembránnal hozunk létre bevonatot a hatóanyagraktár körül (*b*). Hatóanyagot tartalmazó gélmátrix bevonásával képezhető olyan multipartikuláris rendszer, amelynek bevonata a térfogatnövekedés következtében feltagolódik (*c*), és az így nyílt pórusok nyitnak

II. táblázat

Gélállapot változását előidéző fizikai és kémiai ingerek

	Inger	Hidrogél típusa	Mechanizmus
Fizikai	Hőmérséklet	Hőérzékeny hidrogél poly(N-izopropilacrylamid)	Hőmérsékletkülönbség befolyásolja a gél duzzadt állapotát meghatározó polimer-polimer és víz-polimer kölcsönhatásokat.
	Elektromos mező	Polielektrolit hidrogél	A duzzadás elektromos mező hatására módosul, a gélmembrán feltöltődik és a töltéssel rendelkező hatóanyag elektroforetikus mobilitása is változhat.
	Fénybesugárzás	fotokróm (pl. azobenzén) csoportot tartalmazó polimer	A fotokróm csoport konformációs változása befolyásolja a gél duzzadt állapotát.
	Mágneses mező	Mágneses részecskék eloszlatva alginátos mikroszférában	Mágneses tér alkalmazása megváltoztatja a duzzadást illetve a pórusok méretét.
	Ultrahang-besugárzás	Etilén-vinilalkoholos hidrogél	A duzzadás az ultrahang hatására bekövetkező hőmérséklet-emelkedéstől függ.
Kémiai	pH	Savas vagy bázikus hidrogél	A duzzadás illetve a pórusok nyitása vagy zárása a pH-tól függően következik be.
	Ionerősség	Ionos hidrogél	Az ionerősség változás miatt a gélen belüli ionkoncentráció megváltozik és a duzzadás módosul.
	Komplexképző, reaktáns	Elektron akceptor csoportot tartalmazó hidrogél	A duzzadás az elektron donor összetevővel képződött komplexnek tulajdoníthatóan módosul.
	Enzim-szubsztrát	Enzimet tartalmazó hidrogél	Szubsztrát jelenlétében enzimatikus átalakulás, a reakciótermék jelenlétében változik a duzzadás



2. ábra. A hatóanyagleadás intelligens polimerekkel történő szabályozásának elvi lehetőségei

utat a gyógyszer számára. Pórusok nyitásán illetve zárásán alapuló szabályozás kialakítható olyan polimer bevonattal, amelynél a gél zsugorodásakor tágulnak illetve duzzadásakor összehúzódnak a pórusok.

A gélállapot változását fizikai és kémiai ingerek idézhetik elő ***(II. táblázat).***

Hőmérséklet

A hőmérsékletnek mint ingernek az alkalmazása azzal függ össze, hogy a testhőmérséklet kóros állapotban gyakran eltér a normál 37 °C-os értéktől. Hőközléssel könnyen megvalósítható a hőmérséklet külső szabályozása, akár helyi hipertermiás kezeléssel. Ez a megoldás hatékony lehet a rákterápiában [12], a lokális hőmérséklet emelés alkalmazható hőérzékeny hatóanyag-felszabadító rendszerrel kombinálva [13].

A hőmérséklet jelentősen befolyásolja a polimer molekuláris kölcsönhatásait, így a láncok közötti hidrogénhíd kötéseket és hidrofób kölcsönhatásokat, valamint a polimer lánc és oldószer közötti interakciót. A hőmérséklet duzzadást befolyásoló hatása a hidrogél érzékenységétől függ. A hőmérséklet emelkedése pozitív hőmérséklet-érzékenység esetén a duzzadást, negatív hőmérséklet-érzékenység esetén fordított változást, a géltérfogat csökkenését váltja ki. Bizonyos polimergélek esetében a hőmérséklet emelkedése a fázisok elkülönülését okozza, a gél elveszíti folyadéktartalmát. A gél fázisátalakulását jellemző kritikus hőmérséklet (*lower critical solution temperature, LCST*) kopolimerek előállításával változtatható. Például a gyakran vizsgált poli(N-izopropilakrilamid)-ból készített NIPA-gél negatív hőmérséklet-érzékenységet mutat (LCST≈32 °C), ugyanakkor a fázisátalakulás hőmérséklete akrilsavval vagy alkilmetakriláttal történő kopolimerizáció révén módosítható [14, 15].

A hőmérséklet hatóanyagleadást befolyásoló hatása összetett mechanizmussal jellemezhető és az adott gél viselkedésének következményeként ellentétes irányú is lehet [16]. Egyes esetekben a kritikus hőmérséklet fölött (>LCST) a fázisátalakulásból eredően a gél felszínét sűrű „bőrréteg" borítja be, amely a hatóanyag felszabadulását lelassítja, így a felület szabályozó szerepének (*surface regulation*) tulajdonítható nyújtott gyógyszerhatás érhető el. Más esetekben a kritikus hőmérséklet fölött az egész géltömeg olymértékű zsugorodása mehet végbe, hogy az oldott hatóanyag a mintegy kifacsart folyadékkal együtt préselődik ki a gélből (*bulk matrix squeezing*), így a hatóanyagleadás felgyorsul [17].

Elektromos mező

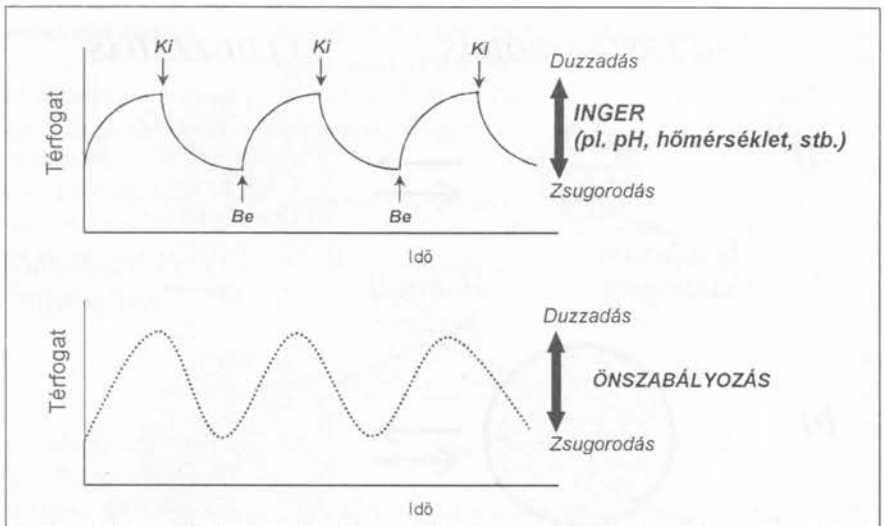
A polielektrolit gélek olyan kereszttérhálós polimerek, amelyek a láncokon ionizálható csoportokat tartalmaznak. Vizes oldatban ezek a funkciós csoportok ionizálódhatnak, a polimer makroiont ellentétes töltésű kisebb ionok stabilizálják. A duzzadás úgy következik be, hogy a gélen belüli magas ionkoncentrációból eredően, ozmózis révén víz áramlik a gélbe.

Elektromos mező hatására, az oldott hatóanyag feltöltött gélmembránon át végbemenő permeációja különböző elektrokinetikus jelenségek alapján értelmezhető illetve

szabályozható [18–20]. Emellett a hatóanyag felszabadulásában kisebb molekulák (pl. pilokarpin) esetén elsősorban a zsugorodó gél folyadéktartalmának kipréselődése játszhat szerepet, nagyobb méretű molekulák (pl. inzulin) felszabadítására inkább a duzzadás ad lehetőséget [21].

A hatóanyag leadása történhet ioncsere útján is, pl. a pozitív töltésű oldott hatóanyag helyét hidrogénionok válthatják fel a gélben [22].

Sikeresen állítottak elő olyan vezetőképességgel rendelkező polimereket, amelyek alkalmasak a neurotranszmitter-felszabadulás elektromos potenciál hatására bekövetkező, „ki-be" mechanizmussal jellemezhető utánzására [23, 24].



3. ábra. Polimergélek hatóanyagleadását szabályozó mechanizmusok elvi vázlata ingervezérelt és önszabályozó térfogatváltozás esetén

Fénybesugárzás

Fényérzékeny polimerek előállíthatók fényérzékeny vegyületek (pl azovegyületek) polimervázhoz kapcsolásával. Ebben az esetben a gél duzzadását vagy zsugorodását a fotokróm csoport fényenergia hatására bekövetkező konformációs vagy konfigurációs változása befolyásolja. Ilyen polimerek felhasználhatók gélekből alkálifémsóknak [25], fehérjéknek [26] és aminosavaknak [27] fotokémiai úton történő felszabadítására.

Mágneses mező, ultrahang

Mágneses ingerre válaszoló gélrendszerek ferromágneses anyagnak a gélbe ágyazásával készíthetők [28, 29]. A hatóanyag felszabadítására mikropórusok jelenléte ad lehetőséget, miközben a leadás sebességét a gél zsugorodása illetve duzzadása szabályozza. A rendszer jól szabályozottan és reprodukálhatóan működik, mert egy adott mágneses erőhöz meghatározott hatóanyagleadási sebesség rendelhető. Egy jövőbemutató elképzelés szerint a mágneses teret egy karóra méretű eszköz szolgáltatná, amellyel a beteg az adagolás idejében egy gombnyomással indíthatja meg a gyógyszer felszabadulását [30].

Hasonlóan jól reprodukálható kísérleteket végeztek ultrahang-besugárzásra válaszoló polimergélek alkalmazásával is [31].

pH

A környezet kémhatására érzékeny polimerek alkalmazását indokolja a szervezet folyadéktereinek jellemző pH-értéke. A hatóanyagleadás szabályozása szempontjából a gasztrointesztinális rendszer mentén változó pH [32] évtizedek óta ösztönzőleg hat különböző pH-érzékenységgel rendelkező polimerek előállítására, amelyeknek hagyományos bevont gyógyszerformákban történő alkalmazásának előnyeiről bőséges tapasztalat áll rendelkezésre [33, 34].

Bár a vérplazma és kötőszöveti folyadék kémhatása jellemzően állandó, a tumoros szövet pH-értéke pl. emlőrákban alacsonyabb [35].

A pH-változás befolyásolja a polielektrolitok ionizáltságát és oldhatóságát, illetve konformációját. A polielektrolit gél pH-érzékenységét alapvetően az ionizálható csoportok típusa és száma, a kereszttérháló sűrűsége és a polimerváz hidrofobicitása határozza meg [35–39].

A környezeti pH-ra válaszoló polimerek alkalmazást nyerhetnek a hatóanyagnak helyspecifikus (pl. colonban) [40] és tumor-specifikus [41] felszabadítására. A pH-érzékeny polimer előnyösen használható fel antacid készítmények formulálásánál is, amikor a gyomor pH változása a polimer mikropórusainak összehúzódásával vagy tágításával szabályozza a hatóanyag kiáramlását [42].

Glükózérzékeny polimergél

Peppas és munkatársai pH-érzékeny kopolimert állítottak elő polimetakrilsav (PMAA) és polietilénglikol (PEG) összekapcsolásával [43]. A féligáteresztő membránnal körülvett gélrendszer belsejébe glükóz-oxidázt ágyaztak, amely enzim a glükózt glükonsavvá alakítja. Megemelkedett vércukorszint hatására az enzim működése fokozódik, ami a környezet pH csökkenését eredményezi. Savas közegben a PMAA karboxil csoportjai protonálódnak, hidrogénkötések jönnek létre a PMAA szekvenciák, illetve a PMAA és a PEG étercsoportja között. Megnő a hidrofobicitás, ami a gél összehúzódását okozza és így a kitágult mikropórusokon keresztül szabaddá válhat a raktározott inzulin, a gélzsugorodás mértékének megfelelően.

Önszabályozó gélek

A hatóanyagot környezeti inger által vezérelt „ki-be" mechanizmus révén felszabadító gélrendszerek mellett előnyös lehet olyan polimerek előállítása, amelyek belső

program által szabályozva (*self-oscillating*) végzik működésüket ***(3. ábra).*** Az önszabályozó polimergélek pl. mint hormon-felszabadító gyógyszerrezervoár-rendszerek kerülhetnek alkalmazásra.

Az önszabályozás megvalósítására alapul szolgálhat a Belouszov-Zsabotyinszkij-reakció [44]. Ennek során, a magasabb oxidációs állapot hidrofilebbé teszi a polimerláncot és a gél fázisátalakulásának hőmérséklete eltolódik. Így a gél térfogatváltozása az oxidációs állapot változásainak megfelelően zajlik. [30, 45, 46]

Összefoglalás

Az összefoglaló második részében a hatóanyagleadás intelligens polimerekkel történő szabályozásának lehetőségeit kívántuk áttekinteni. A szervezet homeosztázisa révén pontosan szabályozott fiziológiás körülmények (pl. hőmérséklet, pH, ionkoncentráció, glukózszint stb.) olyan ingerként szolgálhatnak, amelyeket a gyógyszeres terápia optimálása érdekében célszerű a gyógyszerforma tervezésénél figyelembe venni illetve alkalmazni. Az önszabályozó polimergélekkel végzett kutatások rámutatnak arra, hogy a nemlineáris rendszerek különleges viselkedésének klasszikus kémiai példája, az oszcilláló reakciók modellje is felhasználható új elven működő gyógyszerformák kifejlesztéséhez.

IRODALOM

1. *Rácz, I., Selmeczi, B.:* Gyógyszer-technológia III. Medicina Könyvkiadó, Budapest, 2001. – 2. *Arshady, R.:* Polym. Eng. Sci. *30,* (15), 1746–1758 (1989) – 3. *Oppenheim, R. C.:* Int. J. Pharm. *8,* 217–234 (1981). – 4. *Lonsdale, H. K.:* J. Memb. Sci. *10,* 81–181 (1982). – 5. *Singh ,P., Desai, S. J., Simonelli, A. P., Higuchi, W. I.:* J. Pharm. Sci. *57,* (2), 217–226 (1968). – 6. *Okano, T.:* Biorelated Polymers and Gels. Academic Press, SanDiego, 1998. – 7. *Lemmer, B:* J. Contr. Rel. *16,* 63–74 (1991). – 8. *Santoro, N., Filicori, M., Crowley, W. F.:* Endocrinol. Rev. *7,* 11 (1986). – 9. *Marchetti P, et al.:* Diabetes *43,* 827–830 (1994). – 10. *Peppas, N. A., Gurny R., Doelker E., Buri P.:* J. Membr. Sci. *7,* 241–253 (1980) – 11. *Segot-Chicq, S., Peppas, N. A.:* J. Contr. Rel. *3,* 193–204 (1986) – 12. *Weinstein, J. N., Magin, R. L., Cysyl, R. L., Zaharko, D. S.:* Cancer Res. *40,* 1388–1392 (1981) – 13. *Iga, K., Ogawa, Y., Toguchi, H.:* Pharm. Res. *9,* 658–662 (1992) – 14. *Gutowska, A., Bae, Y. H., Kim, S. W.:* J. Contr. Rel. *22,* 95–104 (1992) – 15. *Bae, Y.H., Okano, T., Kim, S. W.:* Pharm. Res. *8,* 531–537 (1991) – 16. *Hoffmann, A.S.:* J. Contr. Rel. *6,* 297–305 (1987). – 17. *Okano, T., Bae, Y. H., Jacobs, H., Kim, S. W.:* J. Contr. Rel. *11,* 255–265 (1990) – 18. *Grimshaw, P. E., Nussbaum, J. H., Grodzonsky, J. A., Yarmus, M. L.:* J. Chem. Phys. *93,* 4462–4472 (1990). – 19. *Zhao, H., Price, W. E., Wallace G.:* J. Membr. Sci. *87,* 47–56 (1994) – 20. *Yuk, S.H., Cho, S.H., Lee, H. B.:* Pharm. Res. *9,* 955–957 (1992) – 21. *Sawahata, K., Hara, M., Yasunaga, H., Osada, Y.:* J. Cntr. Rel. *14,* 253–262 (1990) – 22. *Kwon, J. C., Bae, Y. H., Okano, T., Kim, S. W.:* J. Contr. Rel. *17,* 149–156 (1991) – 23. *Miller, L. L., Lau, N. K., Miller, E. K.:* J. Amer. Chem. Soc. *104,* 5242–5244 (1982). – 24. *Mirmoshseni, A., Price, W. E., Wallace, G.:* Polymer Gels and Networks *1,* 61–77 (1993). – 25. *Shimidazu, T., Yoshikawa, M.:* J. Membr. Sci. *13,* 1–13 (1983) – 26. *Ishihara, K., Shinohara, I.:* J. Polym Sci. Polym. Let. Ed. *22,* 515–518 (1984). – 27. *Sunamoto, J., Iwamoto, K., Mohri, Y., Koninato, T.:* J. Amer. Chem. Soc. *104,* 5504–5506 (1982). – 28. *Edelman, E. R., Kost, J., Bobech, H., Langer R.:* J. Biomed. Mater. Res. *19,* 67 (1985). – 29. *Zrínyi M., Gács J., Simon Cs–né:* 213 215 sz. Magyar Szabadalom. – 30. *Dagani, R.:* Intelligent Gels. Chem. Eng. News *75,* 1997 június. – 31. *Gurny, R, Junginger, H. E., Peppas, N. A.:* Pulsatile Drug Delivery. WVG, Stuttgart., 1993: p. 48. – 32. *Antal, I.:* Acta Pharm. Hung. *71,* 280–288 (2001). – 33. *Lehmann, K.:* In Aqueous polymeric coatings for pharmaceutical dosage forms McGinity, J. W. (ed.), Marcel Dekker, New York, 1989. p.153–247. – 34. *Cole, G., Hogan, J., Aulton, M.:* Pharmaceutical Coating Technology. Taylor and Francis, London, 1995. – 35. *Van den Berg, A. P., et al:* Oncology *18,* 457–462 (1982). – 36. *Grignon, J., Scallan, A. M.:* J. Appl. Polym. Sci. *25,* 2829–2843 (1972). – 37. *Firestone, B. A., Siegel, R. A.:* Polymer *29,* 204–208 (1988) – 38. *Brannon-Peppas, L., Peppas, N. A.:* Biomaterials *11,* 635–644 (1990). – 39. *Gerheke, S. H., Cussler, E. L.:* Chem. Eng. Sci. *44,* 559–566 (1989). – 40. *Brondsted, H., Kopecek, J.:* Biomaterials *12,* 584–592 (1991). – 41. *Kitano, K., Wolf, H., Ise, N.:* Macromolcules *23,* 1958–1961 (1990). – 42. *Rácz, I., Antal, I., Plachy, J.:* Pharmazie *51,* 323–327 (1996). – 43. *Dorski, C. M., Doyle, F. J., Peppas, N. A.:* Polym. Mater. Sci.Eng. Proceed. *76,* 281–282 (1997) – 44. *Field, R.J., Körös, E., Noyes, R. M.:* J. Amer. Chem. Soc. *94,* 1394–1395 (1972). – 45. *Scott, S. K.:* Oscillations, Waves, and Chaos in Chemical Kinetics. Oxford University Press, New York, 1994. – 46. *Yoshida, R., Takahashi, T., Yamaguchi, T., Ichijo, H.:* J. Amer. Chem. Soc. *118,* 5134–5135 (1994).

I. Antal, M. Mariann: ***Application of intelligent polymers in pharmaceutical technology. Part II: Mechanisms for controlling drug release***

This summary reviews the possibilities for controlling drug release by the use of intelligent polymers. Physiological environments (e. g. temperature, pH, ion concentration, glucose level etc.) are precisely regulated by the homeostasis of our body and they may serve as stimuli which can be applied to optimize drug therapy during dosage form design. Recent results on self-regulating polymer gels demonstrate that principles related to special behaviour of nonlinear systems in the classical model of oscillation reactions also give an opportunity to develope pharmaceutics based on new principles.

Semmelweis Egyetem Gyógyszerészeti Intézet, Budapest, Hőgyes Endre u. 7. – 1092