Vein allograft’s viability during tissuebank storage

PhD Thesis

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1. Introduction

The majority of revascularisation need graft implantation. The disposable conduits not only autogenous vein for infrainguinal bypasses but expansive prosthetic grafts. Consider the results of the international publications on and following the pioneer work of the National Vascular Surgical Institute and take advantage of our regional department arterial and vein homograft transplantation and investigation have to be demanded. Harvesting, storage and implantation of homografts can be possible because of large number of donation, high instrumental and experimental possibilities either of the Regional Tissue Bank nor highly educated members of Department of Vascular Surgery. Connecting with clinical activity the comprehensive cell viability investigation have to be started.

2. The aim of the study

to evaluate the Methyl Tetrazolium (MTT) assay is a reliable, reproducible and simple viability assay for vein homograft studies. In the first part of the investigation a new cost effective, rapid, reproductive and reliable viability test had to be developed which can be easily applicable for the homografts studies. In the second part of the investigation we compared in vitro the viability effects of conventional and routine cryopreservation with the new preservation protocol (-4 °C storage in tissue culture medium)

We would like to widely introduce a new cost-effective therapeutic preservation protocol at the usage of allografts. We examined the viability of vein allografts harvesting from cadavers and multi organ donors by two different preservation protocol. We have to be decided wheater the cryopreservation or the new preservation protocol was effectible.
3. Hipothesis

1. Methyltetrazólium colorimetric assay can be used as an indicator of vein homograft viability
2. The MTT reductase enzyme reaction follows the Michaelis enzyme kinetics
3. During long term refrigerated storage the vein homografts reach the same level of viability as is commonly found in cryopreserved allografts
4. Was there viability index difference between two differently harvested allograft group?
   – Brain dead heart beating donors (no ischemic period)
   – Cadavers (max. 24 hours ischemic period)

5. Was there effect of medium replacement on vein homograft viability

4. Materials and Methods

Fresh intact vein samples were harvested during multi-organ procurement and were tested to investigate the assay. MTT assay is based on the production of purple formazan pigment from methyltetrazolium salt by the mitochondrial enzymes of viable cells. The amount of pigment formed can be detected by spectrophotometry after extraction by methylcellosolve. The reliability of the MTT assay was tested by a fluorescent dye combination (1 µg/ml propidium iodide PI and 4 µM/ml SYTO-16 stains). The enzyme kinetics of the reaction was also investigated because it was unknown in the case of vein homografts. Great saphenous vein (GSV) biopsies had been cryopreserved, and the remained samples were divided into two matched groups and stored in tissue culture medium (TCM) for 42 days at +4°C, either with or without regular medium replacement. Each vein allograft was biopsied and assayed for viability on every third day by the methyl tetrazolium (MTT) reduction assay. Viability indexes of vein allografts harvested from brain-dead multi-organ donors and of cadavers whose warm ischemic period were maximum 24 hours were also compared.
5. Results

The color density is proportional to mitochondrial enzyme activity, reflecting the number of viable cells. The optimal reagent concentration, biopsy size and incubation period were established. There is a linear relationship between the vein homografts weight and the pigment production activity. A 8.6% nonspecific reaction was observed in the negative controls. The MTT cleavage up to 0.1% (w/v) follows the Michaelis kinetics. We calculated the Michaelis constant (2805±130 µM) the maximal velocity (196±2.2x10^{-5} µM sec^{-1}) and the velocity constant (6.98±0.2x10^{-7} sec^{-1}). The viability were also assessed and calculated by a fluorescent dye combination comprising PI and SYTO 16. The PI stains dead cells (red), the SYTO-16 stains live cells (green). The staining can be visualised simultaneously and the live/dead ratio can be calculated by image analysis software. The MTT assay and the staining method were corroborative. Vein allografts stored for 42 days at +4°C showed similar viability (58.9±1.2%) to cryopreserved (59.7±2.3%). This was true even when cryopreserved and thawed allografts was subjected to 3 days post thaw incubation under presumably favorable condition (58.7±1.6%). There was no viability index difference between the medium replaced and non replaced and two differently harvested groups.

6. Conclusion

The use of MTT in colorimetric assays offers high sensitivity. The MTT assay is a simple, non expensive, efficient, reliable and reproducible viability test for vein homograft studies. Long-term storage of vein allografts at +4°C is valuable option for regular banking practice. Sufficient amount can be procured from cadavers similar to the tissue donors.
7. Discussion

The homograft implantation in adults became routine procedure in the last decade, due to improvement of cryopreservation protocol and vascular surgical experience and high age of population. The cryopreservation protocols are well known. The decreasing viability during cryopreservation is too high so it must be solved. In vitro viability test of fresh vein homografts harvested from cadavers and multiorgan donors is unique and no issues in current international literature can be found.

8. Clinical investigation

We compared in vivo the viability effects of conventional and routine cryopreservation with the new preservation protocol ( +4 °C storage in tissue culture medium). Depending on the results of the allografts which were preserved with the new protocol we would like to implant in humans in the case of adequate indications. We would like to widely introduce the new, cost-effective, reliable preservation protocol of allografts stored cold anoxia.
9. Thesis

According to our investigations there are declared:
1. The MTT assay is a simple, non expensive, efficient, reliable and reproducible viability test for vein homograft studies.
2. The MTT reductase enzyme reaction follows the Michaelis kinetics up to 0.1% concentration
3. Vein allografts stored for 42 days at +4°C showed similar viability (58.9 ±1.2%) to cryopreserved (59.7 ± 2.3%).
4. There was no viability index difference between the medium replaced and non replaced and two differently harvested groups.
5. Long-term storage of vein allografts at +4°C is valuable option for regular banking practice. Sufficient amount can be procured from cadavers similar to the tissue donors.

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11. List of publications

**Original articles**


**Abstracts**

1. B. Galambos, T. Gunther, T. László: Experience and results of the treatment of infected vascular grafts; Cardiovascular Surgery, Suppl. 2 ESCVS 50nd congress 2000 abstracts.
5. Cardiovascular surgery ESCVS 52nd Congress abstract Suppl. 2 Vol: 11.


Presentations


2. B. Galambos, T. Gunther, T. László Experience and results of the treatment of infected vascular grafts; ESCVS 50nd kong.; 2000 Budapest; 2 poszter

3. B. Galambos, L. Tamás, P. Zsoldos, T. Czigány, L. Jakab; Vascular injuries in everyday practice ESS 64nd congress; 2001 Budapest; Előadás fődíj

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7. B. Galambos, L. Tamás, P. Zsoldos, T. Czigány, L. Jakab; Tapasztalatok és eredmények a supraaortikus erek sebészeti kezelésében; MAÉT; 2003 Szeged; poszter


