Biomechanical and coagulation aspects in the vascular graft research

Ph.D. Doctoral Thesis

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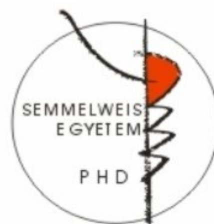

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1. INTRODUCTION

Among the *biological* vascular grafts the routinly used, good quality autogenous vein provides appropriate opportunity to the central and peripheral vascular reconstructions. If there is not enough sufficient quality autogenous graft for surgery, homografts can be a reasonable matter of choice. Some advantages of the vascular homografts are the good biomechanical profile, resistance against the infection, furthermore they can be used in infrainguinal or distal reconstructions. Sufficient number of vascular graft can be collected from cadavers or multi-organ donors, which can be stored and utilized even in another institute later on. Disadvantages are that they can evoke immune response, can degenerate in time, and require proper storage conditions.

It is wildly important goal that graft storage is a long-term feasible, cost-effective and simple process. On the cardiovascular field cryopreservation gained popularity, but this process demands special equipment with high running and service expenses. For these reasons simple storage techniques at 4 °C in cold anoxia have came into view again recently.

In contrast to cryopreservation, storage in cold anoxia is a simple, instrumentally undemanding and therefore a cheaper procedure. Furthermore the intracellular freezing injuries and the cytotoxicity of the DMSO can be avoided. Refrigeration at 4 °C is widely used to reduce the metabolic needs of a tissue/organ before transplantation. The prevention of the intra- and extacellular damage provides maintained viability and biomechanical properties which promote the long-term sufficiency of the implanted grafts.

Galambos et al. investigated the viability of the great sapheonus veins (GSV) with methyl tetrazolim (MTT) assay after 6 weeks storage in tissue culture medium (TCM) at 4°C. They found around 60% viable cells which corresponds to the same level after the cryopreservation.

The most studied properties of the stored grafts are the biomechanical, viability, histological and coagulation aspects, which can be compared with different cells, tissues, grafts based on some sort of standardized method. By current opinion the biomechanical

properties have critical importance at the time of implantation to achive long time patency and graft function.

Nowadays, the most often used *synthetic* grafts in the arterial reconstruction are Dacron® (PET - polyethylene terepthalate) and GORE-TEX® (ePTFE - expanded polytetrafluoroethylene) grafts. Simple availability in the required size and pliableness can be mentioned among their advantages. On the other hand they are quite expensive, act like a foreign body, are liable to infection and occlusion, their biomechanical characteristics differ from physiological, since they are stiff.

Combinated grafts are to combine the advantages of the biological and synthetic grafts. Dacron grafts underwent different impregnations (albumin, collagen, antibiotics, silver). Dacron mesh augmentation of glutaraldehyde fixed umbilical veins are listed here. Mentionable the biological or synthetical frameworks (collagen, cellulose, hydrogel) providing mechanical stability, for smooth mucle and endothelial cell seedings techniques aiming for a superior functional vessel graft. A good exemple to the last mentioned is the bacterial cellulose based vascular graft, which's coagulation and biomechanical properties have been studied lately in Sweden.

Bacterial cellulose (BC) is hydrophilic and it has high water content, 99%, but it is insoluble in water. BC is synthesized and secreted extracellularly by *Acetobacter Xylinum.* During the production process, it is also possible to modify several properties including pore size, surface properties and layering of the material. The mechanical properties of BC tubes are similar to those of pig carotid arteries. It has a compliance curve resembling that of a native artery more than any other synthetic material on the market.

Integration of a material with the host tissue is essential for the success of tissue engineered blood vessels. BC is well integrated into the host tissue and does not induce any inflammatory or foreign body response in animal experiment modell both in subcutaneus and in carotis position. Therefore, it is clear that BC has good biocompatibility and promising potential as scaffold material.

2. OBJECTIVES

I. Morphological and biomechanical analysis of the fresh GSV to be used for coronary bypass operation.

II. The effects of cold strorage (4°C) in sterile normal Krebs Ringer (nKR) solution for 1 and 2 weeks from active and passive biomechanical and mophological point of view.

III. Evaluation of the biomechanical properties after storage in X-VIVO™ 10 tissue culture medium for 1,2,3,4 weeks and comparison with samples stored in nKR.

IV. Cryopreserved and thawed GSV segments biomechanical alteration in comparison with cold stored (4°C) grafts.

V. Systematical assesment of the histological changes under different storage conditions and duration. In addition to evaluate the marcromolecular permeability we examined the transmural diffusion of colloidal iron (Ferrlecit®).

VI. Comparison of the procoagulation properties of BC as potential vascular graft and the most commonly used sythetic vascular graft materials based on a new method.

3. METHODS

3.1 Collection of saphenous vein segments and their storage

Saphenous vein samples were collected routinely from patients scheduled for coronary bypass operations. Segments remaining after the operation, and longer than 40 mm, were used in this study. The programme was approved by the Ethical Committee of the Semmelweis University (123/2006). A total of 72 venous segments from 32 patients were tested. Segments were randomly distributed into eight groups:

- Fresh segments - those tested immediately after the operation **/fresh,n=14/**
- Cold storage (4°C) in normal Krebs-Ringer (nKR) for 1 week **/nKR-1week, n=10/**
- Cold storage (4°C) in normal Krebs-Ringer (nKR) for 2 weeks **/nKR-2weeks, n=10/**
- Cold storage (4°C) in X-VIVO™ 10 TCM for 1 week **/Xvivo-1week, n=8/**
- Cold storage (4°C) in X-VIVO™ 10 TCM for 2 weeks **/Xvivo-2weeks, n=8/**
- Cold storage (4°C) in X-VIVO™ 10 TCM for 3 weeks **/Xvivo-3weeks, n=8/**
- Cold storage (4°C) in X-VIVO™ 10 TCM for 4 weeks **/Xvivo-4weeks, n=8/**
- Cryopreserved for 4 weeks and thawed segments **/cryo, n=8/**

We used physiological salt solution (nKR) as transport medium generally, and in 2 groups as storage solution, furthermore, it was used as organ bath solution during mechanical tests. The physiological salt solution (nKR solution) we applied was: 119 NaCl, 4.7 KCl, 1.2 NaH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 24 $NaHCO_3$, 5.5 glucose and 0.02 ethylenediaminetetraacetic acid (EDTA) (measures in units of mmol/l).

As TCM, the X-VIVO™ 10 medium solution (BioWhittaker, Walkersville, MD,US, BW04380Q) was applied. It is a serum-free, chemically defined TCM, containing recombinant human proteins (albumin, transferrin and insulin), phenol and gentamicin as antibiotic. It has been successfully applied for the sensitive task of maintaining proliferating bone-marrow progenitor cells.

For specimens kept in sterile glass vials in nKR and the X-Vivo solutions, the temperature was stabilised at 4 °C and contact with air was restricted with rubber caps.

Segments selected for cryopreservation were immersed in Ringer-lactate solution (containing antibiotics) and, as cryo-protectant, dimethyl sulphoxide (DMSO) was added gradually up to a concentration of 10%, and the equilibrated for 20 minutes at room temperature. Freezing was also achieved gradually: initial freezing rates of 1 °C/min were increased to 5 °C/min after reaching -40 °C. The specimens were then stored for some weeks in the evaporation of liquid nitrogen ensuring temperatures of -140 to -150 °C. The specimens were thawed by employing a fast technique: they were simply immersed in nKR solution thermostated at 37 °C.

3.2. In vitro biomechanical test

Any adhering connective tissue was removed by careful preparation, side branches, if any, were tied up. The segments were then immersed in thermostated (37°C) nKR solution, bubbled with 95% O_2 and 5% CO_2 in the tissue chamber of the in vitro pressure angiometer, cannulated at both ends. Their length was uniformly extended by 10% to mimic in vivo axial extension. The intraluminal pressure could be controlled by a system including a pressure reservoir and a continuous infusion withdrawal pump (Harvard Apparatus, Holliston, MA, USA). The middle portion of the segment was visualised by video-microscopy using a Leica microscope, a Philips analogue video camera and a Cole-Palmer fiber optic illuminator unit. The segment's photograph was formed on the monitor screen. Inner and outer diameters could be measured by the manual setting of two calliper spots at the contours of the segment. Calibrations were done with a Wild micrometer calibration Etalon piece.

Segments were pre-incubated at 10 mmHg for 30 min, and then the pressure was increased in a stepwise manner (in steps of 7.5 mmHg) from 0 up to 85 mmHg. A time period of 2 min was allowed for equilibration at each pressure level. These measurements were repeated with 10 mM norepinephrine in the bath to induce maximum smooth-muscle contraction. Then, Ca^{2+}-free Krebs-Ringer solution was applied, and the pressure steps was repeated up to 150 mmHg. Finally, as a specific tensile strength testing, 300 mmHg intraluminal pressure was established and the presence of any leaks was checked.

3.3 Biomechanical computations

The measurement of inner diameter required the specific setting of the light pipe; these measurements were not made at each pressure step. On such occasions, the inner diameter was computed from the value of the outer diameter supposing isovolumetry of the wall material.

Tangential stress was computed applying the Frank-Starling equation:

- $\sigma = p * r_i / h$

where p is the intraluminal pressure, r_i is the inner radius and h is the wall thickness

Incremental distensibility was computed by:

- $D_{inc} = \Delta V/(V * \Delta p)$

where D_{inc} is incremental distensibility, ΔV is the lumen volume change in response to an intraluminal pressure change of Δp and V is the initial volume.

Incremental elastic modulus was determined by using the equation:

- $E_{inc} = 2r_i^2 * r_o/(r_o^2 - r_i^2) * (\Delta p/\Delta r_o)$

where r_i and r_o are the actual values of inner and outer radii and Δr_o is the outer radius change in response to the pressure change Δp. The contraction of the segments was evaluated at each pressure level as percentage change of the inner diameter from the fully relaxed value.

Statistical comparisons were made with one- and two factor analyses of variances (ANOVAs). In certain cases, the significance level of the correlation coefficient was also evaluated. Values of p below 0.05 were accepted as significantly different.

3.4 Histological assessment

As a supplement to the biomechanical analysis routine histological evaluation was carried out of the different stored saples at the Department of Forensic Medicine. The grafts were fixed in 10% buffered formaline, thereafter paraffin embedding and sectioning to 4µm thin specimen, were preformed. For the morfological analysis the sections were stained with common laboratory stains:

- *Hematoxylin-eosin (HE) staining*
- *PTAH (**p**hospho**t**ungstic **a**cid **h**aematoxylin) staining*
- *Trichrome staining (Mallory's staining)*
- *Orcein staining*

Beyond the morphological evaluation we proposed to examine the vascular tissues integrity after different storage conditions. Thus we used colloidal iron diffusion to study the vascular permeability which is an important functional parameter of the grafts. Ferrlecit® (sodium ferric gluconate complex in sucrose injection) is a stable macromolecular complex used to replete and maintain the total body content of iron. It has an apparent molecular weight on gel chromatography of 289,000 - 440,000 daltons.

Specimens from the different groups were incubated in physiological saline, containing 25% Ferrlecit® for 60 minutes at room temperature. After physiological saline rinsing specimens were fixed in 10% buffered formaline, as mentioned above.

- *Perls' Berlin blue reaction*

According to our hypothesis there might be a correlation between the diffusion rate and the progressive tissue disintegration and membran injury during the storage. Therefore, the Berlin blue reaction might demonstrate different patterns due to different storage conditions.

3.5 Measurements of coagulation on bacterial cellulose and conventional vascular grafts, generation of the thrombin and factor XIIa

The development of novel non-thrombogenic biomaterials or surface modifications to attenuate thrombogenicity, especially for synthetic vascular grafts, has remained one of the main challenges in biomaterials science. Despite more than 30 years of intense research, there is still no consistently reliable polymeric-based synthetic graft, especially for applications less than 5 mm internal diameter. The most commonly used materials for vascular grafts are expanded poly(tetrafluoroethylene) (ePTFE) and poly(ethyleneterephtalat) (PET). The primary mechanisms of graft failure are intimal

hyperplasia due to compliance mismatch between the stiff bypass graft and the elastic artery, poor blood flow propagation through the grafts caused by the currently used stiff materials and surface thrombogenicity.

The aim of this study was to establish new methods to compare the pro-coagulant properties of vascular graft materials and to use these methods in a comparison of a novel vascular graft material made of bacterial cellulose (BC) with existing commercially available graft materials of PET (Dacron®) and ePTFE (GORE-TEX®). We used thrombin generation assay, modified to detect material (surface) induced coagulation, and a novel imaging method visualizing the coagulation process.

Tubes of BC, ePTFE and PET were opened longitudinally. Round pieces of 7 mm Ø were punched out and fitted into 96-well plates with the luminal side facing upwards. Samples were fixated with O-rings to the bottom of wells. Thrombin generation experiments were performed as previously described by *Hemker et al.* but excluding the tissue factor and phospholipid activator. Briefly, 80 µl aliquots of PFP were transferred to the prepared 96-well plate and 20 µl PBS was added. All material samples were run together with an individual thrombin calibrator containing 80 µl plasma and 20 µl reconstituted Thrombin Calibrator (Thrombinoscope BV, The Netherlands). As a control, O-rings without materials were used. To initiate thrombin generation, Fluo-buffer (Thrombinoscope BV) was mixed with the fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland) in DMSO and 20 µl immediately transferred to each well. Three replicate wells were used for each measurement. The fluorescence (ex: 390 nm, em: 460 nm) was measured with a Fluoroscan Ascent plate reader (Thermo Electron Corporation, Land) every 20-s for 100 min. The thrombin generation parameters' lag time (the time for thrombin generation to start), endogenous thrombin potential (ETP), peak (maximum rate of thrombin formation), and time-to peak (ttpeak, the time to were the peak thrombin generation rate occurs), were analysed using the Thrombinoscope software (Thrombinoscope BV). The generation of activated factor XII at the material surfaces was measured on materials prepared in the same way as for thrombin generation. The factor XIIa sensitive fluorogenic substrate Boc-Gln-Gly-Arg-AMC

(Bachem, Bubendorf, Switzerland) in DMSO was mixed with citrated PFP and added to the wells. Four replicate wells were used for each measurement.

Imaging of coagulation was used to visualize and study the activation and propagation phase of coagulation induced by the three tested graft materials thereby enabling surface comparison.Tubes of BC, ePTFE and PET were opened longitudinally. Pieces were cut to fit along one wall of PMMA spectrophotometry cuvettes (Kartell, Italy) with the luminal surface outwards, and held in place by the custom-made poly-lysine coated clips. The cuvettes were placed in a custom-made holder and illuminated from beneath with white-light-emitting LEDs.

Time-lapse images of all materials were simultaneously captured and converted into video sequences using our own software. Coagulation was detected in the video as an increase in light intensity attributed to light scattering from the insoluble fibrin network. The captured images were analyzed using custom-made software that calculates the coagulation time of the plasma at the surface (surface coagulation time) and in the bulk (propagation).

4. RESULTS

4.1 Biomechanical alterations of the great saphenous vein.

Active and passive biomechanical changes were registered and evaluated during the different storage procedures. Storage in the cooled physiological salt solution characteristically induced a dilation of the morphological (relaxed) diameter of the segments (*Fig.1a)* with no alteration in wall thickness (*Fig.2a).*

The morphological dilation of *nKR* stored vs. fresh segments, also the reduction of lumen of cryopreserved vs. fresh segments was significant $p<0.001$. During storage in X-VIVO™ 10 solution the lumen is practically unaltered in weeks 2-4. The inner radius of controls was 1.70 ± 0.12 mm, measured at 10 mmHg pressure, increased to 1.98 ± 0.15 mm at the end of the storage period in nKR. (*Fig.1a)*

There was a moderate but statistically significant reduction in isobaric distensibility ($8.9 \pm 1.3 \times 10^{-3}$ vs. $4.9 \pm 1.9 \times 10^{-3}$ 1/mmHg), *(Fig. 2b)* the elastic modulus did not change *(Fig.2c)* The ability to contract in response to maximum concentrations of norepinephrine was almost fully lost at the end of the first week (1.6 ± 0.8% from 10.1± 1.5% of fresh controls, $p<0.01$).

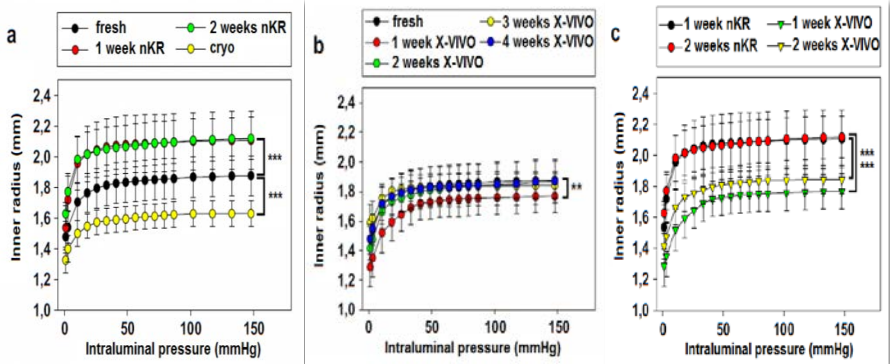

Figure 1.

Comparison of intraluminal pressure vs. inner radius plots of human saphenous vein segments stored under different conditions. Relaxed state of smooth muscle. ***a.*** *Fresh vs.1 or 2 weeks 4 °CnKR stored, and cryopreserved.* ***b.*** *Fresh vs.1,2,3 or 4 weeks 4 °C X-VIVO™ 10 stored* ***c.*** *Stored in 4 °C nKR and4 °C X-VIVO™ 10 stored specimenss. two-way ANOVA tests, *p < 0.05, **p < 0.01, ***p < 0.001.*

After thawing and incubation, the *cryopreserved* segments had decreased lumen and increased wall thickness 586± 56 µm vs. 408 ± 17 µm of controls, ($p<0.01$), relaxed values measured at 10 mmHg intraluminal pressure *(Fig. 1a, 2a)* . Their elastic properties were maintained and their ability to contract was not entirely lost *(Fig. 3).*

Segments stored in the *TCM* differed significantly in their biomechanical properties from those stored in the nKR solution. There was an initial loss of lumen diameter, which was in contrast with the morphological dilation of the Krebs-stored segments *(Fig. 1b and 1c)*. It is remarkable that the passive pressure-radius plots after 2 weeks of storage were almost identical with plots of fresh segments *(Fig. 1b*).

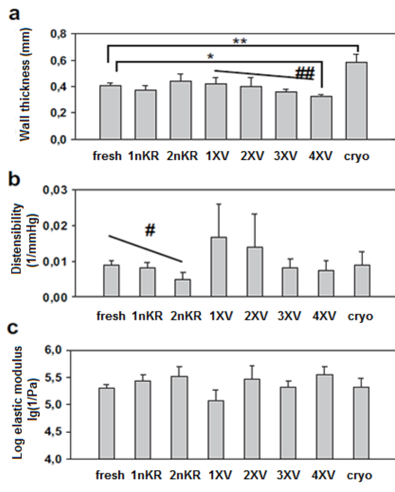

Figure 2.
Comparison of geometric and elastic properties of human saphenous vein segments stored under different conditions.

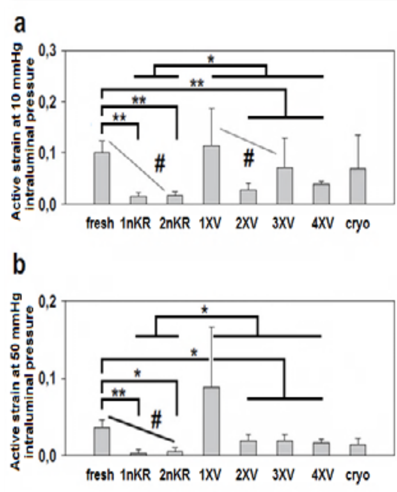

Figure 3.
Active strain (relative reduction of inner diameter due to contraction from fully relaxed state) of human saphenous vein segments stored under different (cooled) conditions.

In these segments, in contrast to the elevated wall thickness of the cryopreserved segments, the wall thickness continuously decreased with increasing storage time (the significance level of the correlation coefficient was $p<0.01$).

In variance with Krebs-stored segments, neither of the elasticity changes reached the level of statistical significance. One remarkable difference was the relative preservation of the ability to contract: although there was a continuous reduction in contractility, the X VIVO™ 10 preserved segments showed a significantly higher contractility than their Krebs-stored counterparts (*Fig. 3a and 3b)*, $p<0.05$ for both 10 and 50 mmHg intraluminal pressure levels.

4.2 Histological results

Morphological analysis perfomed on fresh venous grafts, segments stored in 4°C nKR for 1 and 2 weeks, cryopreservered and thawed samples, and specimens stored in 4°C TCM (X-VIVO™ 10) for 1,2,3 and 4 weeks.

Vein segments showed notable individual differences, in most cases intimal layer expansion were observed even with fresh segments. Venous wall revealed some degree of fibrotic changes from the beginning. The morphological assessment implemented in different storage grops with each staining described before, caracteristic tissue signs developed indicating vessel wall injury depending on storage time. Moreover, we could follow the damage, fragmentation of the elastic fibers in a time dependent manner. *Fig.4* demonstrates staining on fresh grafts which applied to the microscopic evaulation.

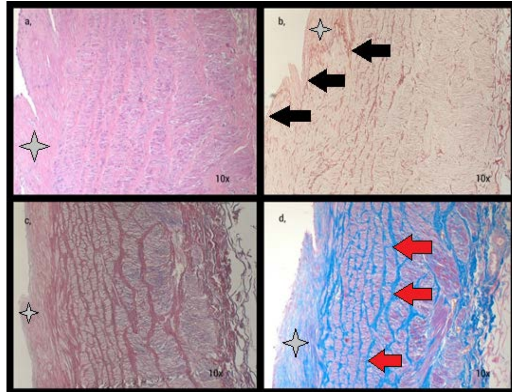

Figure 4.
Fresh great saphenous vein (longitudinal) sections from the same patient. Applied staining are HE (a), orcein (b), PTAH (c) and trichorme (d), 10x magnification ✧wide intimal layer, 🡐 irregular internal elastic membrane. 🡐 fibrotic changes in the tunica media.

On histological examination the endothel coverage (tuncia intima), the internal elastic membrane, the smooth muscle layer (tunica media) have been estimated, and the grade of the intramural oedema defined.

Fresh samples generally showed focal mild expansion of the tunica intima. In one case even a recanalising plaque was observed. The internal elastic membrane was one layered with waved

morphology, and smooth cell layer was also maintained. We found increased amount of elastic fibers in some cases and there was a mild grade of intramural oedema. Berlin blue reaction could be sporadically demonstrated.

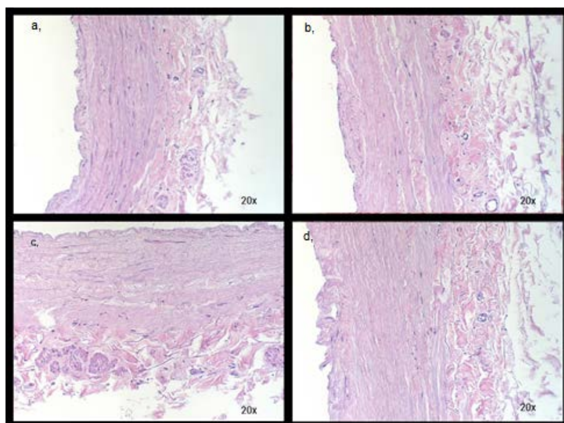

Figure 5.
Great saphenous vein sections from the same patient stored inX-VIVO™ 10 for 1 week (a), 2 weeks (b), 3 weeks (c), 4 weeks (d), HE staining 20x magnification.
In correlation with the storage time the media homogenize, the number of the cell nucleus decreses, but there is mild oedema during the whole storage time.

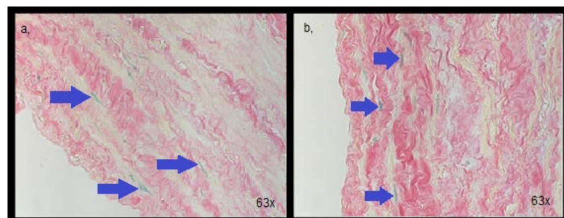

Figure 6.
Positive Berlin blue reaction marked → in the intimal layer and in the luminal section of the tunca media.
63x magnifying.

Samples, stored in nKR solution for 1 or 2 weeks showed focal mild or moderate expansion of the tunica intima. The elastic membran presented normal one layered, waved morfology after the first week, but 2 weeks storage induced fragmentation. The structure of the tunica media loosened and elongated, the grade of oedema was mild or morderate. There was positive Berlin-blue reaction in most of the samples: after one week storage dye certified in the intimal layer, and appeared even in the deeper smooth mucle layer in samples stored for 2 weeks in nKR.

Cryopreserved and thawed segments showed irregular concentric widening of the intimal layer, and in some section fibrotic

plaque were presented. The internal elastic membran showed sporadical fragmentation, and the structure of the tunica media became odematous, and there was mild fibrosis between the smooth muscle cells.

In specimens stored in X-VIVO™ 10 TCM the intimal layer showed unaltered focal mild expansion after 1,2,3 or 4 weeks of cold storage. The internal elastic membrane showed waved morfology and sporadical fragmentation after the first and second storage week. After the third and fourth week the elastic plate showed fading and became hardly defineable. In correlation with the storage time the tunica media homogenized, and showed pale staining. The number of the cell nucleus decreased, but there was mild oedema during the whole storage time. There was positive Berin-blue reaction in most of the samples. *(Fig 5.)*

Based on the evaluation of the 8 storage groups there are notable individual structural differences between the vein segments. Typical pathological changes of chronic venous disease often observed in the tunica initma and tunica media. Storage in both nKR and X-VIVO medium resulted intimal expansion and fragmentation of the internal elastic membrane.The oedematous transformation of the tunica media was more representative in nKR than X-VIVO storage.

We gathered controversial results in the assessment of the macromolecular permeability. Satining after iron colloid diffusion showed positiv Berlin-blue reaction even in some of the fresh segments, which indicates the loss of barrier funcion in some cases at the beginning of the storage. Specimens stored in nKR showed positive staining in deeper and deeper portion of the smooth muscle layer in time suggesting a higer rate of the transmural diffusion. This finding represents a tendency, but the low number and diversity of the samples does not allow any quantitative evaluation. *(Fig 6.)*

In the remaining storage groups any (reproductible) conclusive positive Berlin blue reaction coudn't be registered.

4.4 Measurements of coagulation on bacterial cellulose and conventional vascular grafts.

Both ePTFE and BC were found to generate significantly longer lag times ($p<0.001$, n=13) than PET. The ttpeak values demonstrated the same effects, and both ePTFE and BC had significantly reduced lag times (ePTFE: $p<0.01$, BC: $p<0.001$,n=13) as compared to the PET graft material. Furthermore, the BC graft material was found to demonstrate lower thrombin generation peaks than both PET and ePTFE, with a significance level of $p<0.001$ (n=13). Low but significant differences were also found between the peak of thrombin generation of PET and ePTFE ($p<0.05$, n=13) where ePTFE generated the highest peaks. In ETP, the only statistically significant difference found was between PET and BC, and only at a low significance level ($p<0.05$, n=13). It should be noted that variations between donors caused substantial deviations in the measurements *(Fig. 7. and 8.)*, especially affecting the thrombin generation peak and ETP.

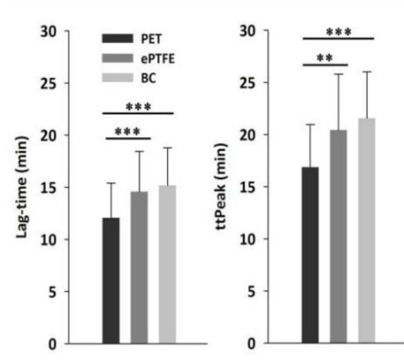

Figure 7.
Thrombin generation experiments were performed in PFP on the exposed lumen of PET, ePTFE and BC graft material samples.

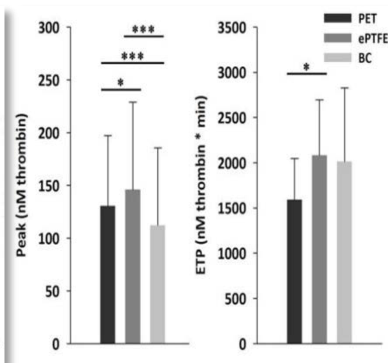

Figure 8.
Thrombin generation experiments were performed in PFP on the exposed lumen of PET, ePTFE and BC graft material samples.

By samples were secured using heparinized O-rings the mean rate of fluorescence increase was calculated over a 60 min period after the start of the test. *(Fig. 9a.)* The PET graft material facilitates a generation of thrombin approximately five times faster than both ePTFE ($p<0.001$) and BC ($p<0.001$). No significant difference between ePTFE and BC was found.

Factor XIIa, the initial enzyme in the intrinsic pathway, was measured at the surface of graft materials PET, ePTFE and BC, using a similar approach as for the thrombin generation measurements. The results showed that the PET graft material surface activates factor XII at the highest rate.

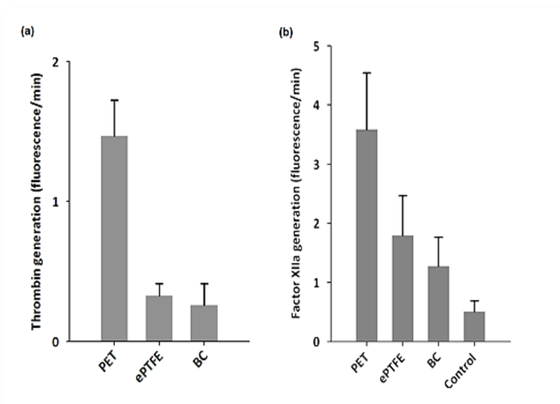

Figure 9.
*Thrombin **(a)** and factor aXII **(b)** generation rate at the material surfaces of PET, ePTFE and BC calculated from the average fluorescence increase rate measured over 60 min.*

The ePTFE ($p = 0.011$) and BC ($p = 0.001$) materials demonstrate considerably lower activation rates than PET. *(Fig. 9b.)*

Imaging of coagulation was used to visualize and study the propagation phase of coagulation induced by the three tested graft materials. Representative time-lapse images of the coagulation process at the material samples are presented in *Fig. 10.*

All time-lapse images are colour-coded to visualize the fibrin network density. It is evident from the images that PET generated a rapid onset of coagulation at the material surface as compared with both ePTFE and BC. The ePTFE activated coagulation at a lower

rate than BC, but in contrast it had a very fast propagation phase that spread the coagulation throughout the bulk plasma rapidly.

The initial coagulation at the material surfaces was measured and calculated as surface coagulation times and are presented in *(Fig. 11a)* (n = 6). The order in which surface coagulation was detected at the surface was, in increasing order (mean ± SD): PET (27 ± 8 min) < BC (46 ± 9 min) < ePTFE (61 ± 21 min).

The propagation phase of coagulation was also analysed and is presented in *(Fig. 11b)*, where the mean propagation rate was measured in four consecutive 0.5 mm intervals from the material sample (n=6). These results revealed dramatically faster propagation from the ePTFE material than from both PET and BC materials.

A novel method facilitating imaging of coagulation in addition to thrombin and factor XIIa generation was used to evaluate the impact of different vascular graft materials on activation of coagulation in plasma. Three graft materials, including two well-known, commercially available materials, PET (Dacron®) and ePTFE (GORE-TEX®), as well as a novel graft material, constructed from bacterial cellulose, were all tested in this experimental setup.

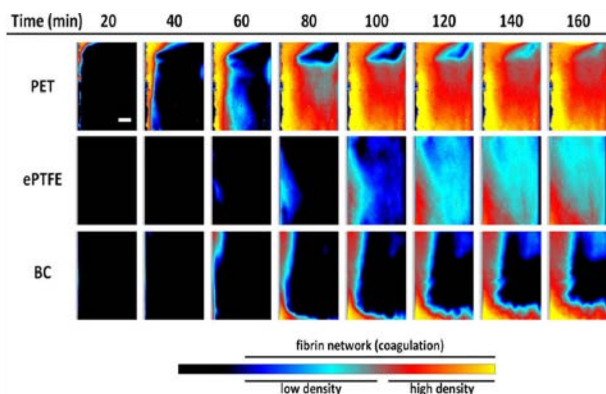

Figure 10. *Representative time-lapse images from imaging of coagulation tests in PFP at the exposed lumen of PET, ePTFE and BC graft samples.*

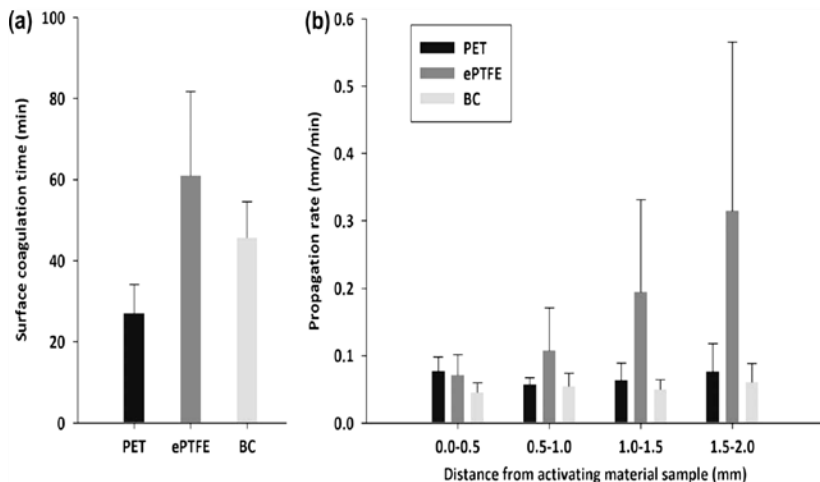

Figure 11.

Imaging of coagulation.

(a) Surface coagulation times for PET, ePTFE and BC from imaging of coagulation experiments.(b)Propagation of coagulation is presented as mean propagation rates measured in four consecutive 0.5 mm intervals from the PET, ePTFE and BC graft material samples.Values are presented as means + SD. Plasma from six different donors was used.

Reproducible data could be demonstrated in throbin and factor XIIa generation studies and in visualisation experiments, wich certifies the high accurancy of the methods. BC as a biomaterial has a similar coagulations profile to ePTFE which is promising.

Present available methods studying the coagulation process, measure the coagulation in the bulk without regards to the initiation point of the coagulation or the kinetics describing the propagation from the initiation point. This technique allows us to visualize and define the exact point where the coagulation is initiated and how coagulation propagates, highly relevant questions in the study of material interactions with blood.

5. CONLUSIONS

This study investigated one of the most important biologic vascular prosthesis, the human great saphenous vein. We studied the biomechanical alteration after different storage conditions lasting different times. Altogether, 72 vein segments remaining from CABG of 32 patients were collected and divided into eight groups: (1) fresh, (2) stored in 4°C nKR solution for 1 week, (3) stored in 4°C nKR solution for 2 weeks, (4) stored in 4°C TCM for 1 week, (5) stored in 4°C TCM for 2 weeks, (6) stored in 4°C TCM for 3 weeks, (7) stored in 4°C TCM for 4 weeks, (8) cryopreserved samples. When stored in nKR (normal Kerbs-Ringer solution), we found dilated segmental morphology, decreased distensibility and these segments lost their ability to contract in a week. In contrast, TCM (X-VIVO™10)-stored vein segments maintained their contractility for a week, which decreased only slowly afterwards (2,3,4 weeks). Their lumen diameter did not alter in 4 weeks of storage time and elastic parameters of these segments were similar to the fresh segments. Cryopreserved samples exhibited thickened wall, decreased lumen diameter, and contractility. Due to its advantageous biomechanical profile the TCM stored great saphenous vein could be a feasible alternative for different vascular reconstructions in absence of autogenous vein. Long-term studies are necessary to investigate the potential clinical applicability.

For measurements of biomaterial-induced coagulation we have adapted the automated calibrated thrombin generation method to compare BC with clinically used graft materials i.e., expanded poly(tetrafluoroethylene) (ePTFE) and poly(ethyleneterephtalat) (PET). We have also visualized the propagation of coagulation on biomaterial surfaces. Thrombin generation experiments revealed dramatic differences between the materials tested. Both ePTFE and BC were found to generate longer lag times and ttpeak values than PET. Most importantly, BC was found to generate the lowest ‘‘peak”, indicating a slower coagulation process at the surface. These results are also supported by the measurements of factor XIIa generation and analysis of surface coagulation times, which were detected in the following increasing order (mean ± SD): PET (27 ± 8

min) < BC (46 ± 9 min) < ePTFE (61 ± 21 min). In our experimental setup reproducible data could be collected in throbin and factor XIIa generation studies and in visualisation experiments, which certify high accurancy of the methods. BC as a biomaterial has a similar coagulations profile to ePTFE which is promising.

Real-time measurement of coagulation seems to has the potential to become a powerful tool for evaluation of biomaterials for blood-contacting devices.

6. NEW FINDINGS

I. According to the literature we found significante differences in the biomechanical and histological apperance of the freshly harvested VSM segments.

II. Great saphenous vein segments stored in TCM (X-VIVO™ 10) at 4°C maintain a substantial level of vascular contractility up to 4 weeks, in contrast, segments stored in physiological saline (normal Krebs-Ringer solution) for 2 weeks show a slight morphological dilation, decreased distensibility and total loss of smooth muscle contractility.

III. There is no significant difference in point of the biomechanical profile between the TCM cold stored great saphenous vein segments and the cryopreserved and thawed ones. They are therefore substitutable with each other in certain cases.

IV. Consecutive morphological analysis proved the slow disintegration of the vascular tissue, particularly the extacellular components, which was mainly influenced by the duration of the storage but not the storage medium.

V. In the evaluation of the surface coagulation properties on surgical biomaterials (vascular grafts) both the ELISA-plate adapted spectrophotometric method, and the visualization method in the PMMA cuvettes revealed a sensitive, reproducible, fast method. Real-time measurement of coagulation has the potential to become a powerful tool for evaluation of biomaterials.

Publications related to the dissertation

1. **Molnár GF,** Kékesi V, Nádasy GL, Nemes A, Monos E. Alteration in the biomechanical properties of the great saphenous vein during preservation under different conditions. Érbetegségek 2013;20(3):55-59.

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3. Fink H, Faxälv L, **Molnár GF**, Drotz K, Risberg B, Lindahl TL, Sellborn A. Real-time measurements of coagulation on bacterial cellulose and conventional vascular graft materials. Acta Biomater. 2010;6(3):1125-30.