EUROPEAN CLINICAL STUDY FOR THE APPLICATION OF REGENERATIVE HEART VALVES - ESPOIR

Axel Haverich

Department of Cardiothoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany

Objective

Acquired and congenital heart disease can necessitate heart valve replacement. However, current heart valve substitutes are not considered ideal as they need anticoagulation, bearing the risk of bleeding when manufactured from non-organic material, or they degenerate when they are derived from animals or humans thereby leading to frequent reoperation especially in the young population**.**

Materials and Methods

Decellularised fresh homografts, which have shown promising early results in pulmonary valve replacement in children and young adults, could potentially avoid significant activation of the immune system, as more than 99% of donor DNA is removed during the decellularisation process.

Results

In May 2002, the first human implantation was performed in Chisinau, Moldavia. Since then 91 decellularised only valves have been implanted using the Hannover protocol (66 in the last 4 years). 71 were implanted in the pulmonary position of patients with a mean age of 15.3 ± 10.1 years, a median of 13.5 years, and a range of 0.13- 50.6 years. No explantation for degeneration has been performed so far. Matched comparison (age, type of congenital defect, number of previous operations) to bovine jugular veins and conventional cryopreserved homografts showed superior results not only for freedom from reoperation, but also for the rate of grafts with degenerative signs.

Conclusions and Future Work

These early clinical results have indicated that the decellularised homografts have a potentially superior performance compared to conventional cryopreserved homografts in the pulmonary position. These auspicious results have convinced the European Commission to fund a unique project. Starting in 2013 the ESPOIR consortium will undertake a prospective multi-centre trial to include at least 200 patients from 8 leading European Centres for Congenital Heart Surgery, for robust statistical evaluation of DHV in direct comparison to conventional pulmonary valve substitutes.

Acknowledgements

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COSMETIC APPROACH TO HEART VALVES TISSUE ENGINEERING

Gino Gerosa and Filippo Naso

Dept. of Cardiac, Thoracic and Vascular Sciences, University Hospital of Padua, Italy

Heart valve substitutes suffer important drawbacks and none of currently commercially available prostheses provide satisfactory long-term durability, ideal hemodynamics and remodelling performance. The attempts of Tissue Engineering for fabrication of substitutes using biological or biodegradable scaffolds, coupled with the manipulation of cells in diseases tissues (Cells Therapy) has enabled, in the mid-2000s, the development of the Regenerative Medicine approach in which the regeneration of damaged tissues is permitted by their substitution in addition to the stimulation of the body's own repair mechanisms in order to heal previously irreparable injuries [Mason 2008]. Inspite of the fact various scientists reported promising results, Regenerative Medicine of heart valves (RMHV) is still at the beginning of its evolution without any significant clinical translation. In the 2000 Hoerstrup *et al.* produced the first functional trileaflet heart valves made from a bioabsorbable polymer (polyglycolic-acid PGA) and seeded with ovine myofibroblasts and endothelial cells. The construct was implanted in pulmonary position in lambs with a maximum follow-up of 20 weeks. This synthetic scaffold was improved by Sutherland *et al.* (2005) with the addition on the mesh of poly-L-lactic acid. The graft, seeded with ovine mesenchymal stem cells, was implanted in sheep reaching a follow-up of 8 months. Up to date this result can be considered the best obtained combining the use of cells and synthetic scaffold [Gottlieb D 2010,Weber B 2011,Emmert MY 2012]. In the early 2000s the use of biological scaffolds for RMHV began with the occurrence of dramatic clinical events such as the death of paediatric patients implanted with a Synergraft engineered porcine heart valve [Simon 2003], high failure rate of bovine derived vascular grafts [Spark JI 2008] and several clinical re-intervention due to the immunological reaction of the heart valve substitute Matrix P [Rüffer A 0210].These events highlighted the need of safer biological substitutes with more exhaustive preclinical study on using animal models [Hopkins R 2009, Jordan 2012, Weymann 2012, Gallo 2012].The longest follow-up reported in literature was 15 months, and it was obtained by our research group. During the last 10 years there has been a consistent improvement in cell culture and development of new polymers, but this was coupled with an evident regression of the clinical translation of heart valve constructs. Beautiful images of *in-vitro* scaffold repopulation as well as of cell lineage differentiation as a consequence of pre-conditioning in ultra-modern bioreactors, collide with the basic requirement of reliable, safe and effective clinical application. However, biological scaffolds for HVRM provided the best results in preclinical studies. Additionally biological tissue meets important requirements allowing both to maintain several trophic factors (crucial for proper homing of cellular components) and a great time saving not requiring bioreactor pre-conditioning or cellular pre-seeding.

References

Mason C *et al.* Regen Med 3:1–5, 2008.
Hoerstrup SP *et al.* Circ 102:III-44-III-4, 2000.
Sutherland FW *et al.* Circ 111:2783-91, 2005.
Gottlieb D *et al.* J T Card Surg 139:723-31, 2010.
Weber B *et al.* Eur Heart J 32:2830-40, 2011.
Emmert M *et al.* JACC Card Interv 5:874-83, 2012.
Simon P *et al.* Eur J Card Surg 23:1002-06, 2003.
Spark JI et al. Br J Surg 95:582–85, 2008.
Rüffer A *et al.* Eur J Cardioth Surg 38:78-85, 2010.
Hopkins RA *et al.* J T Card Surg 137:907-13, 2009.
Jordan JE *et al.* J T Card Surg 143:201-08, 2012.
Weymann A *et al.* Circ J 77:207-216, 2012.
Gallo M *et al.* Artif Organs 36:E138-50, 2012.

COMMERCIAL EXPLOITATION OF CARDIOVASCULAR TISSUE ENGINEERING PRODUCTS

Michael Harder

Corlife OHG, Hannover, Germany

The medical need

Currently, there is a high medical need for novel therapeutic options for heart valve replacements, cardiac muscle to restore infarct regions, substitutes for coronary arteries and other blood vessels such as venous valves, and reconstruction of congenital heart defect. Tissue engineering (TE) is a promising technology addressing these needs. However, and for the time being, TE products are very expensive and therefore suitable only for a few conditions.

Regulatory Pathways

TE products are highly regulated. A sophisticated and sometimes confusing network of European Directives and Regulations [1-4], as well as national laws and guidelines, impose high demands on the authorities and companies. Simplifications are expected by an increasing number of common legal concepts and rules, such as e.g. labeling. However, as of today, national and regional rules impede the free exchange of TE products in Europe.

The conflict between altruistic donation and commercial exploitation

The first step in the manufacture of any allogeneic TE product is the donation of a tissue. Such donation is always an altruistic one, given to a non-for-profit procurement organization [1]. This requirement must be met to avoid a human tissue trade, which is banned in the European Union. However, a significant investment has to be dedicated to cover the costs for the development, approval, manufacture, quality control and the distribution of TE products. This investment is carried by private companies, who expect a fair return for their capital. An open and fair partnership between procurement organizations, TE companies and healthcare organizations can help to avoid potential ethical conflicts.

Cost structure and cost coverage

TE products are made in small quantities and mainly upon request. Due to the high demands on safety and quality, clean rooms and qualified personnel must be dedicated. This leads to a fixed cost structure, which in turn results in high production costs.

Future Trends

In the foreseeable future, TE products will be available for only a few surgical interventions, for which the national health care systems will provide cost coverage. In this context, health care assessment tools will play a major role in evaluating the risks of investing in novel TE products. However, there is a need to revise the current procedures for allocation of and the reimbursement for TE products [5] in order to prevent a 'two-tier medicine' system.

References

[1] Directive 2004/23/EC of the European Parliament and of the Council of 31st March 2004;

[2] Commission Directive 2006/17/EC of 8th February 2006;

[3] Commission Directive 2006/86/EC of 24th October 2006;

[4] Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13th November 2007;

[5] World Health Organization, 63rd World Health Assembly, Document A63/24 of 25th March 2010, Item 11.21: Human organ and tissue transplantation.

TRANSLATIONAL RESEARCH IN VASCULAR TISSUE ENGINEERING: A CLINICIAN-SCIENTIST'S PERSPECTIVE

Shervanthi Homer-Vanniasinkam

Leeds Vascular Institute, Leeds General Infirmary, Leeds, United Kingdom

Vascular disease (coronary, cerebral and peripheral arterial) continues to be the leading cause of major morbidity, and mortality, worldwide. Whilst this was initially a disease of the Western hemisphere, it is now a global entity, and hence, there are increasing efforts to seek lasting solutions, in terms of revascularization strategies for occlusive vascular disease

Over the years, the vascular surgical community has been well served by autologous (mainly veins) bypass conduits, assisted on occasion by the use of synthetic grafts and patches. However, there has been a growing need for a biological alternative when autologous material is either not available eg the vein has been previously utilised, or is unsuitable for clinical use (varicose, or fibrosed/ thrombosed). Furthermore, when an infected prosthesis or patch is explanted, a biological solution is required for repair and revascularisation

The first report of a biosynthetic tissue engineered graft was by Weinberg and Bell in 1986. Since this publication, there have been several groups who have published their work on a range of scaffolds, and cellularisation techniques. In recent years, some of these conduits and vascular patches have been implanted clinically, thereby fulfilling the 'translational' promise in this field of research

In this presentation, the following aspects will be covered:

1. The areas of clinical need where vascular tissue engineering could provide potential solutions;
2. A brief description of the scaffolds and techniques from the published literature
3. Clinical data on implanted conduits and patches (including complications following implantation);
4. Going forward: the continuing challenges and opportunities in vascular tissue engineering.

ACELLULAR CARDIOVASCULAR TISSUE SCAFFOLDS FROM CONCEPT TO CLINIC

Eileen Ingham[1] and John Fisher[2]

Institute of Medical & Biological Engineering. [1]School of Biomedical Sciences; [2]School of Mechanical Engineering, University of Leeds, Leeds, UK.

Introduction

There is a clinical need for regenerative biological grafts for several cardiovascular applications including cardiac valve replacements, replacement of small and large diameter blood vessels and blood vessel repair post endarterectomy. We have developed methods for the decellularisation of human donor and porcine: aortic and pulmonary, valves, blood vessels and pericardium. An overview of the research, development and progress towards clinical translation will be presented.

Methods

Porcine and human donor tissues were decellularised using a process (Booth et al., 2002) based upon sequential treatment with: hypotonic tris buffer (HTB; 10mM Tris pH 8.0, 0.1% (w/v) EDTA, 10KIU aprotinin), 0.1% (w/v) SDS in HTB, nuclease treatment and sterilisation in 0.1% peracetic acid. Analysis methods included histology, immunohistochemistry, extraction and quantification of total DNA from different areas of the tissue, PCR of DNA to detect functional genes, biochemical assays for GAG and collagen content, in vitro contact and extract biocompatibility assays (Wilcox et al., 2005). The acellular porcine tissues were tested for the presence of residual alpha gal epitope by antibody absorption assays. The acellular tissues were subject to biomechanical evaluation including uniaxial tensile testing, pulsatile flow testing (Korossis et al., 2005), burst pressure testing and suture pull-out tests as appropriate. Following development of bioprocesses, the tissues were subject to in vivo biocompatibility tests in rodents (Mirsadraee et al., 2006) and then large animal proof of concept studies.

Results

The decellularisation processes resulted in excellent removal of DNA from the tissues (Wilshaw et al., 2012) as illustrated in Table (1) for human donor cardiac valves. In general decellularisation lead to some loss of GAGs with retention of the biomechanical properties of the tissues as shown in Figure (1) for porcine pulmonary valves. The tissues were biocompatible in vitro and in vivo. The acellular porcine tissues were devoid of alpha-gal epitope.

Valve	Wall	Junction	Leaflet	Muscle
Aortic	99.2	99.5	97.6	99.1
Pulmonary	99.4	98.1	94.3	99.1

Table (1) Percentage DNA removal from different areas of the human donor cardiac valves

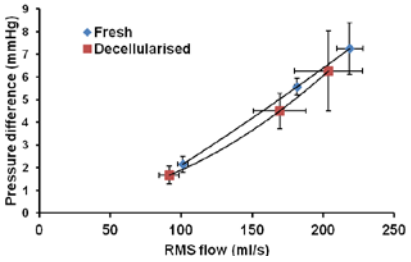


Figure 1: Average pressure difference versus average root mean square (RMS) flow for the fresh (n=6) and decellularised (n=6) porcine pulmonary roots. Mean ± 95 % C.I.

Discussion

Acellular porcine pericardium has been commercialised marketed in Europe as the dCELL® Vascular Patch. Acellular porcine aortic valves have shown regenerative capacity in juvenile sheep and acellular porcine pulmonary valves and blood vessels are currently undergoing long term animal trials. The processes will subsequently be translated to the UK NHS Blood & Transplant (Human tissue) or industry for development of the manufacturing process and clinical studies

References

1. Booth et al. JHVD, 11, 457-462, 2002.
2. Korossis et al. JHVD. 14; 408-422 , 2005.
3. Mirsadraee et al. Tiss Eng, 12:763, 2006
4. Wilcox et al. JHVD 14; 228-237, 2005
5. Wilshaw et al. Tiss Eng A 18; 471-483 (2012).

BIOLOGICAL VASCULARIZED MATRIX (BIOVAM) - SCAFFOLD DEVELOPMENT AND ASSESSMENT

Andres Hilfiker[1], Birgit Andrée[1], Tibor Horvath[1], Marco Lux[1], Letizia Venturini[2], Igor Tudorache[1], Serghei Cebotari[1], Axel Haverich[1]

[1] Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Dept of Cardiothoracic, Transplantation and Vascular Surgery and [2] Department of Haematology, Haemostaseology, Oncology and Stem Cell Transplantation, Medical School Hannover, Hannover, Germany

Introduction

Tissue engineering is a promising technique for reconstruction of failing organs. Based on its size the supply of cells with nutrients and oxygen in constructs requires an *in vitro* and/or *in vivo* vascularization e.g. a biological vascularized matrix (BioVaM). Here we report the generation of a decellularized matrix with a preserved vessel bed for the engineering of 3D artificial tissues. To generate artificial tissues for "proof of principle" experiments in a large animal model, the BioVaM was re-cellularized with porcine primary cells. The influence of residual surfactants on cell survival is discussed.

Methods

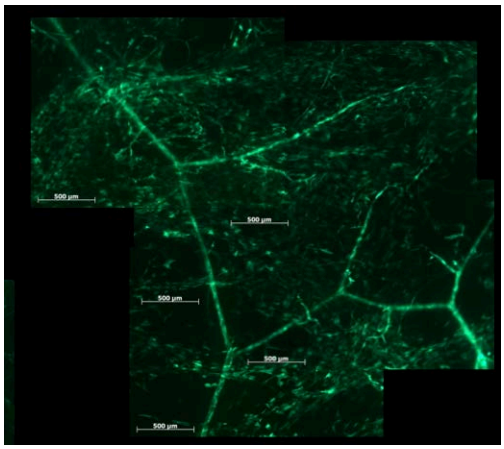
A decellularization process utilizing the surfactants Triton X-100 and sodium dodecyl sulfate (SDS) was used to generate a matrix with preserved pedicles derived from porcine small intestine. The decellularized matrix was intensively washed with PBS. The arterial and/or venous vessel bed was re-cellularized with lentiviral transfected primary porcine cells or a rat heart derived endothelial cell line (RHE). The re-seeded matrix was cultivated for 2 weeks under static and/or perfused conditions. Residual SDS was quantified in the supernatant of decellularized BioVaMs by a methylene blue assay. The critical concentration of SDS was determined for different cell types in cell culture experiments.

Results

Cell survival in the BioVaM was dependent on the cell type used. Complete re-cellularization was achieved with RHE and porcine smooth muscle cells. Primary porcine aortic endothelial cells perished after 6 days of cultivation. In the supernatants of BioVaMs, residual SDS was detected at a concentration of approximately 10 mg/l. Additional cell culture experiments revealed that the maximal SDS concentration tolerated by different cell types was 10 mg/l for primary porcine aortic endothelial cells and 50 mg/l for RHE cells.

Conclusion

SDS is broadly applied in decellularization protocols, but its high affinity to proteins makes efficient removal difficult. Although BioVaMs were intensively washed after decellularization, residual SDS was detected in the supernatant of the decellularized tissues. In cellular assays different cell types have a heterogenous tolerance to SDS, primary endothelial cells being the most sensitive. This is reflected in divergent re-cellularization efficiencies of the BioVaM depending on the cell type used. In summary, monitoring of SDS removal to generate high quality decellularized matrices is mandatory. Moreover, therapeutically relevant cell types should be applied for functional tissue re-cellularization rather than using robust cell lines often used in „proof of concept" studies.



pSMC after 7d culture in BioVaM

GUIDED FUNCTIONAL RE-ENGINEERING OF THE MITRAL VALVE LEAFLETS

Lucrezia Morticelli[1], Daniel Thomas[1], John Fisher[1], Eileen Ingham[1] , Sotirios Korossis[2]

[1]Institute of Medical and Biological Engineering, University of Leeds, United Kingdom;
[2]Department of Cardiothoracic, Transplantation and Vascular Surgery Hannover Medical School, Germany

Introduction

Mitral valve regurgitation represents the second major valvular disorder in the western world, whereas current strategies for mitral valve reconstruction are imperfect [1]. The aim of this study was to develop a TE substitute for mitral valve leaflet reconstruction using acellular porcine pericardium seeded with porcine mesenchymal stem cells (pMSC).

Methods

Porcine pericardial scaffolds were decellularised as described previously [2]. pMSC were cultured on the mesothelial surface of the scaffolds (3 cm diameter) under static conditions, using 3 different cell densities (2×10^4, 1×10^5 and 2×10^5 cells/cm^2). The seeded scaffolds were analysed by scanning electron microscopy (SEM), H & E staining and live/dead staining at 1, 3 and 7 days (Table 1). Following 3 days of static culture, samples seeded with 1×10^5 cells/cm^2 were cultured dynamically (10% strain) for 1 day in a biaxial strain bioreactor (Fig. 1). Following dynamic conditioning, the samples were assessed for cell viability with live/dead staining and MTT assay, and for extracellular matrix (ECM) integrity with H&E (Fig. 2).

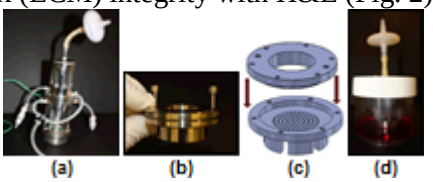


Figure 1: Images of the bioreactor station (a) and tissue loaded on the tissue holders (c, d) and cultured in the culture tubs (d).

Results

The optimum seeding density for acellular pericardial samples was 10^5 cells/cm^2. Samples seeded with this density and maintained statically for 3 days, prior to dynamic conditioning, showed the best cell penetration without a significant disruption in the ECM (Fig. 2). Seeded samples conditioned dynamically for 1 day showed similar levels of viable cells to seeded samples cultured statically for 1 day (Fig. 3 & 4). Cell alignment was also obvious in the dynamically conditioned samples.

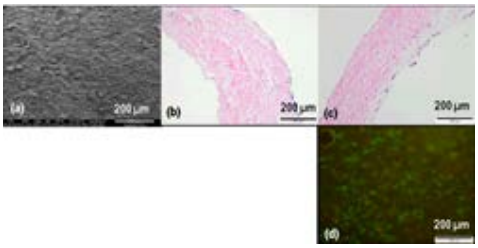


Figure 2:SEM (a), H&E (b, c) and live/dead (d) of scaffolds seeded with pMSCs at 10^5 cells/cm^2 for 24 h (a), 3 (b) and 7 days (c, d).

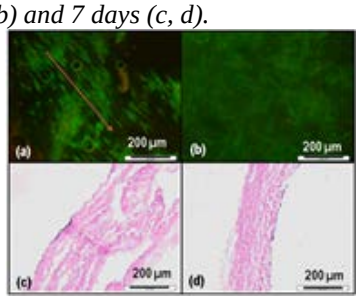


Figure 3:Live/dead (a, b) and H&E (c, d) of seeded samples cultured for 1 day dynamically (a, c) and statically (b,d).Arrow indicates cell directionality.

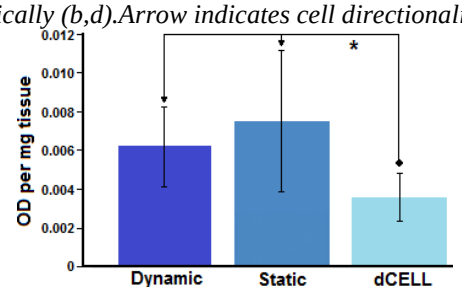


*Figure 4: Cell viability of seeded pericardial scaffolds cultured dynamically and statically after 1day. Unseeded dCELL pericardium used as - control. Means ± 95% C.I., n=4. * indicates significant difference.*

Discussion

Acellular pericardium was shown to be an optimum material for cell repopulation. Reseeded scaffolds were viable after 1 day under 10% dynamic strain. This study provided the basis for optimising the mechano-stimulation of cell-seeded pericardial scaffolds *in vitro* in order to generate heart-valve like tissue.

References

1. Chockalingam *et al*, J Heart Valve Dis, 13(1), 2004.
2. Fisher J *et al*, Matrice decellularisation. Patent PCT/GB02/02341, 2000.

BIOINSPIRED ANISOTROPIC NANOFIBRILLAR MATRICES FOR HEART VALVE ENGINEERING

Adrian H. Chester[1], Jerome Soheir[1,2], Magdi H. Yacoub[1]

[1] Heart Science Centre, Imperial College London, UK, [2]Laboratory for Tissue Biology and Therapeutic Engineering, UMR5305, LYON, France

One great promise of heart valve tissue engineering, especially for children, is the production from autologous cells of an artificial tissue that can resume proper functionality and growth once implanted. In this context, an important goal is to devise a suitable biomimetic scaffold that supports proper cell-matrix interactions and cell growth by reproducing the specific anisotropic fibrillar structure of valves extracellular matrix (ECM).
The success of a tissue engineered heart valve is dependent on developing the right structure, the right interactions between the cells and the right matrix and mechanical force. Different materials have been used as scaffold to obtain the best result. However there are still problems in terms of long term durability.
Our objective is to evaluate a novel type of highly porous anitoropic nanobrillar matrices with regards to structure, mechanical properties and ability to support human adipose derived stem cell (ADSC) colonization, growth and ECM production in vitro.
Nanofibrillar structures were obtained by jet-spraying poly (ε-caprolactone) dissolved in chloroform on a variably rotating drum. Morphological evaluations of the structures were performed using scanning electron microscopy while porosity was calculated from polymer density, weight and volume.
Polymer spraying on rotating drum allowed the formation of nanofibrillar structures (600 nm in average diameter). While spraying on a static drum resulted in isotropic fibre orientation, increasing drum rotation speed up to 3000 rpm increased significantly fibres alignment. In correlation to fibres anisotropy, the scaffolds Young's modulus was simultaneously increased when measured respectively longitudinally and orthogonally to fibre alignment (figure 1). Interestingly, fibre alignment further increased scaffolds porosity.
Rotary seeded matrices resulted initially in cells attached on both scaffold sides but anisotropic matrices allowed a more extensive cellular invasion than isotropic scaffolds, possibly linked to their higher porosity and therefore open structure. Over culture, cells proliferated extensively and bridged the entire scaffolds thicknesses over 10 days. After 18 days, an extensive cellular invasion in all scaffolds type, possibly linked to the high porosity, was evident. Proliferation was up to twice higher within nanofibrillar structures as compared to collagen scaffolds. This study indicates the potential of highly porous anisotropic nanofibrillar matrices as substrate for ADSC in view of tissue formation. In conjunction with their anisotropic mechanical properties, these structures could be of interest for heart valve engineering.

TEXTILE ENGINEERING – A MULTI-SCALE TOOLBOX FOR (CARDIOVASCULAR) TISSUE ENGINEERING

Stefan Jockenhoevel[1,2], Valentine Gesché[1], Robin Ross[1], Kathrin Kleinsteinberg[1], Lisanne Rongen[2], Lena Thiebes[2], Christian Cornelissen[2,3], Petra Mela[2]

[1] Institute for Textile Engineering, RWTH Aachen, Germany; [2]Applied Medical Engineering, Helmholtz Institute Aachen, RWTH Aachen, Germany; [3]Department of Cardiology, Pneumology, Angiology and Intensive Care, University Hospital Aachen, Germany

Introduction

The biomechanical properties of the human body are mainly defined by fibre structures, like e.g. collagen bundles, elastic fibres, fibrin fibres, fibrous cartilage, and ligament etc.. The aim of tissue engineering is to replace, repair, maintain and/or enhance tissue function. Herefore the classical tissue engineering requires an ideal combination of cellular component, scaffold materials and biomechanical and/or biochemical signals. The material plays a central role with regard to the 3D structure, the cell-to-cell-interaction and the biomechanical properties of the complete construct.

Methods

Textile Engineering offers a multi-scale toolbox for the development of scaffold structures on (1) the molecular level of polymer science and biochemical functionalisation, (2) the nano/micro-scale level of fibre production (e-spinning, melt-, wet-, dry-spinning) and on a (3) meso/macro-scale level for the production of 2D and 3D structures by weaving, knitting, braiding etc.

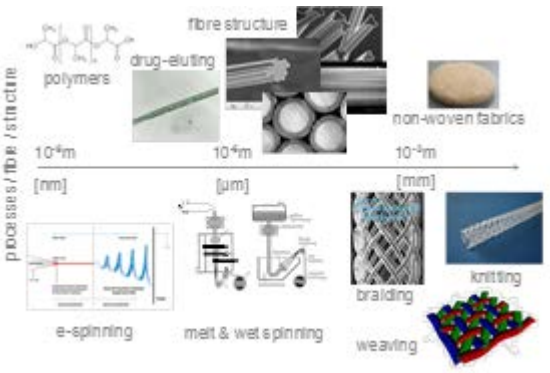


Figure 1: Multi-scale toolbox of textile engineering for the production of scaffold structures

Results

Different textile technologies have been successfully evaluated as single technology or in combination with regard to cardiovascular and pulmonary implants like:

- Non-wovens as scaffold material for heart valve tissue engineering[1]
- Warp-knitted structure for textile-reinforcement of biological vascular grafts[2] and stents[3]
- Braided structures for endobronchial stenting
- Combination of single-fibre-placement and e-spinning for biomimetic, textile-reinforced heart valve prosthesis[4]

Summary

A variety of textile-based and textile-reinforced cardiovascular and endobronchial implants have been developed in our group. The presentation will give an overview about the different textile technologies and their impact for tissue engineering in general and cardiovascular tissue engineering specifically.

References

1. Schmidt D *et al*. J Am Coll Cardiol. 56(6): 510-20, 2010.
2. Koch S *et al*, Biomaterials, 31(17):4731-9, 2010.
3. Weinandy S *et al*. Tissue Eng Part A, 18(17-18):1818-26, 2012.
4. Ross R *et al*. Biomed Tech (Berl). 2012

SOLVING THE PROBLEM LAYER BY LAYER: DESIGNING SCAFFOLDS FOR CARDIOVASCULAR TISSUES

Birgit Glasmacher[1], Oleksandr Gryshkov[1], Alexander Kern[1], Rieke Kortlepel[1], Benjamin Krolitzki[1], Marc Müller[1], Alexandros Repanas[1], Holger Zernetsch[1], Dimosthenis Mavrilas[1,2]

[1]Institute for Multiphase Processes, Leibniz University Hannover, Germany
[2]Laboratoy of Biomechanics & Biomedical Engineering, University of Patras, Greece

Introduction

In Europe, almost 50 % of all mortality cases are related to cardiovascular diseases, with arteriosclerosis playing a major role [1]. A promising way to repair diseased vessels is to implant electrospun tubular scaffolds seeded with autologous cells. While mimicking the structure and the function of the native tissue, this method also avoids foreign body reactions induced by common synthetic grafts. Because of their intention to provide structural support for the cells, the mechanical properties of these scaffolds have to be in a physiological range. Furthermore, to achieve good seeding efficiency, the material used has to be highly biocompatible.

Methods

With our custom-made electrospinning setup, we fabricated multi-layered scaffolds (Fig. 2) with a specific inner and outer layer to improve cell compatibility and infiltration. These layers were spun out of a polymer blend consisting of polycaprolactone, polylactide and polethyleneglycol. The two layers were separated by a hydrophobic layer of poly-caprolactone, which was intended to minimise the leakage in highly porous grafts.

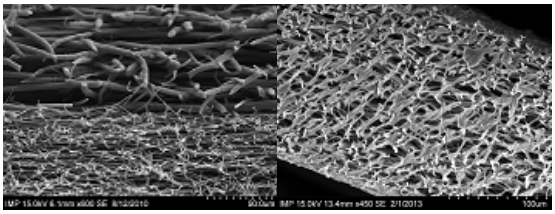


Figure 1: SEM image of multi-layered electrospun scaffold (600x, left) and of the wall thickness of a tubular scaffold (450x, right).

All scaffolds were spun on a rotating mandrel collector ($Ø_i$ 4 mm, 1000 rpm) by using an electric field strength of 66 kV/m and a spin duration of 3 min per layer. Fibre diameters and pore sizes for the inner and outer layers were examined by SEM. A dynamic tensile testing system (BOSE, Electroforce) was used to run cyclic sinusoidal mechanical tests (0 – 10 % strain, 1 Hz, dry conditions and room temperature) on ring samples ($Ø_i$ 4 mm, width 5 mm). Local strain and force were measured for 60 cycles and the recorded data was used to calculate stress/strain values, hysteresis and Young's modulus.

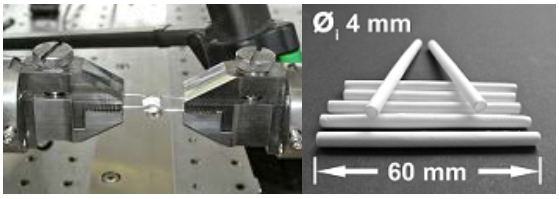


Figure 2: Dynamic tensile testing setup with mounted ring sample (left). Multi-layered electrospun tubular scaffolds (right).

Results

According to preliminary results, the multi-layered scaffolds had a wall thickness of 195 µm. Furthermore, no differences between inner and outer layers were observed for mean fibre diameter (2 µm, n=120) and the pore size (85 µm², n=50). Young's modulus, determined for the circumferential direction, showed a mean value of 15 MPa (n=5). Hysteresis, based on stress and strain values of the loading and unloading phase, was calculated to be 49 %.

Discussion

Regarding the preliminary results our electrospinning setup is suitable to fabricate standardised multi-layered tubular scaffold with desired mechanical properties. Further experiments with electrospun scaffolds (Peel Test, longitudinal testing) and comparative studies with native tissue should prove the potential of the multi-layered grafts. Moreover studies on *in vitro* degradation of the electrospun grafts are in planning.

References

1. European Cardiovascular Disease Statistics 2012 edition, European Heart Network and European Society of Cardiology, September 2012