"Towards Tissue engineering of free standing small diameter branching vessels"

F.Bianchi , C Domenici, G. Vozzi, A Ahluwalia.*

Interdepartmental Centre of Research E. Piaggio, University of Pisa, Pisa, Italy and Institute of Clinical Physiology, CNR, Pisa, Italy.*

Background

Cardiovascular disease (CVD) is one of the principal causes of death in the western world. A great amount of effort is being addressed towards the understanding of the pathological processes involved in CVD as well in the reconstruction, both in vivo and in vitro, of vascular tissue. It has in fact been recognised that the generation of neo- vasculature in vitro is of enormous strategic importance in the treatment of a wide range of vascular related pathologies.

Methods

In this work, microfabrication techniques were combined with fractal algorithms to realise polymeric scaffolds resembling capillary networks. Our objective was to determine if the topological cues from a microfabricated network structure could enhance endothelial cell vitality and tubules formation, if cells were also provided biochemical cues from other cells in co-culture, from angiogenetic promoters and from use of different growth factors, and evaluate the relative importance of each cue.

Although seemingly disorganized, the branching structure of the vascular network is known to follows a fractal geometry. In order to simulate capillary network, a fractal algorithms was developed and integrated into the software of a deposition system, developed at the Interdepartmental Research Center "E.Piaggio"[1].With this technique, tree-like structures, with different fractal dimensions, were made, using bioerodable polymer such as Poly-lactide and polycaprolactone. The scaffolds were seeded with human endothelial cells in co-culture with human fibroblasts; gelatin coated TCP were used as controls. In order to enhance the angiogenic process the cell culture medium was added with different types of growth factors, such as: VEFG. The tubules formation was visualised by staining for PECAM-1, after 15 days from seeding.

Results and conclusions

The results demonstrate that the presence of a structured adhesive scaffold as well as fibroblasts in co-culture contribute to the promotion of a high density and metabolically active network. Tubules formation is still random and it will necessary to promote capillary development along polymer fractal scaffolds. However these structures have several possible applications for example in vascularised tissue engineering and therapeutic angiogenesis.

[1] Vozzi G., A. Previti, D. De Rossi, A. Ahluwalia "Microsyringe based depositino of 2 and 3-D polymer scaffolds wiyh a well defined geometry for application to tissue engineering" Tissue Engineering, 8, 6 (2002)

IN VIVO ATTENUATION OF MYOINTIMAL HYPERPLASIA USING TRANSFORMING GROWTH FACTOR β3 IN AN INTERPOSITION GRAFT MODEL

Murphy MO, Ghosh J, Baguneid MS, Khwaja N, Halka AT, Turner N, Kielty CM, Ferguson MW, Walker MG

Department of Vascular Surgery, Manchester Royal Infirmary and UK Centre for Tissue Engineering, University of Manchester, UK

Background:
The TGFβ family of cytokines exert pleiotropic actions on smooth muscle cell (SMC) phenotype, proliferation and extracellular matrix synthesis. This in vivo study assesses the use of TGFβ3 in attenuating the development of para-anastomotic myointimal hyperplasia in an animal model of small diameter vascular grafts.

Methods:
Under general anaesthesia 10 adult goats underwent bilateral Polyurethane interposition graft insertion in the carotid position. Following completion of the anastamosis each artery received sub adventitial infiltration of 50ng of TGFβ3 around the anastamosis, the other side a placebo. Post-operatively, each animal received 150mg aspirin daily. The arteries were explanted, half at 6 weeks and the remaining five at 3 months for histological examination.

Results:
Vessel wall thickness surrounding the anastamosis was reduced by 30% in TGFβ3 treated arteries compared to placebo at 6 weeks and 3 months, principally due to reduced SMC proliferation. Total collagen content was not significantly different between TGFβ3 and placebo sides. A reduction in total elastin content was found around the TGFβ3 treated side ($p=0.003$). The number of elastin lamellae was lower around the TGFβ3 treated anastamosis that the placebo side ($p=0.042$).

Comments and Conclusion:
Direct, single dose sub-adventitial infiltration of TGFβ3 following insertion of an interposition graft reduces SMC proliferation and elastin content. It would appear that TGFβ3 holds promise as a prophylaxis against the development of myointimal hyperplasia the predominant cause of graft failure in peripheral bypass surgery.

IN VIVO h-VEGF$_{165}$ GENE TRANSFER IMPROVES EARLY ENDOTHELIALISATION AND PATENCY IN SYNTHETIC VASCULAR GRAFTS

Mika Lahtinen MD [1], Vitas Zemgulis MD, PhD [2], Pontus Blomberg MD, PhD [3], Giedrius Baliulis MD [4], Fredrik Carlsson MSc [5], Harry Khamis PhD [6], David Bergqvist MD, PhD [1]

[1] *Department of Surgical Sciences, Section of Surgery, Uppsala University Hospital, Sweden*
[2] *Department of Cardiothoracic Surgery, Uppsala University Hospital, Sweden*
[3] *Clinical Research Center, Karolinska Institutet, Huddinge University Hospital,,Sweden*
[4] *Department of Cardiovascular Surgery, Southampton General Hospital, UK*
[5] *Department of Genetics and Pathology, Uppsala University, Sweden*
[6] *Statistical Consulting Center, Wright State University, Dayton, OH, USA*

Background;
Synthetic vascular graft performance is inferior to autologous vein grafts. This study investigated if 1) local administration of plasmids encoding for human vascular endothelial growth factor (pNGVL3-VEGF$_{165}$) or h-VEGF$_{165}$/FGF-2 combination or 2) graft design affect synthetic graft endothelialisation and patency.

Methods;
Rat abdominal aorta (n=132) was replaced with 60 microns ePTFE graft and pNGVL3-VEGF$_{165}$, pNGVL3-VEGF$_{165}$/FGF-2 combination or control plasmid were administered. In rabbits (n=90), polyester, 60 microns ePTFE, hybrid ePTFE, or 30 microns ePTFE grafts and pNGVL3-VEGF$_{165}$ or control plasmids were used. Rats were sacrificed after 1, 2, 4 or 77 weeks and rabbits after 2, 4, 12 or 43 weeks. In dogs, 60 microns ePTFE (n=10) or polyester grafts (n=10) were inoperated to carotid and hybrid ePTFE grafts (n=15) to femoral position, followed by injection of pNGVL3-VEGF$_{165}$ or control plasmid. Dogs were sacrificed at 6 weeks. Endothelialisation was determined in the midgraft area with scanning electron microscopy (SEM).

Results;
pNGVL3-VEGF$_{165}$ enhanced endothelialisation at every time point in rats, whereas plasmids encoding h-VEGF$_{165}$/FGF-2 combination had worst outcome at 1 week (NS), 2 weeks (p=0.01) and at 4 weeks (p=0.02). In rabbits, polyester grafts had a trend for faster endothelialisation (p=0.08) and pNGVL3-VEGF$_{165}$ enhanced endothelialisation at 2 weeks (p=0.06) but the effect reversed at 4 weeks (p=0.03). pNGVL3-VEGF$_{165}$ improved hybrid ePTFE patency in dogs (p=0,103).

Conclusions;
Improved early endothelialisation and patency with local administration of pNGVL3-VEGF$_{165}$ reveal a new concept for enhancement of synthetic vascular graft performance with gene therapy.

Tissue Engineered Blood Vessels: Production of Extracellular Matrix Components

Maricris Esguerra, Sepideh Heydarkhan-Hagvall, Gisela Helenius, Rigmor Söderberg and Bo Risberg

Vascular diseases are the leading cause of adult death in the western world. Treatment of vascular failure includes bypass surgery or blood vessel replacement with synthetic or autologous vessels. Synthetic materials are not optimal as grafts in small blood vessels and for various reasons many patients lack reserve veins. Tissue engineered blood vessels (TEBVs) may offer a better option to the conventionally used grafts. The ECM has important roles in vessel architecture, strength and function, and is therefore necessary to consider when constructing vessel grafts.
In the present study, we hypothesized that cells in TEBVs, created of isolated vascular cells grown on tube formed polyglycolic acid scaffolds for six weeks, produce their own ECM components but in much less amounts compared to native blood vessels.
Total RNA and protein from TEBVs were extracted and analyzed with Real Time PCR and Western blot respectively. Vessel histology was analyzed with Immunohistochemistry.
The analyses showed production of the ECM components fibronectin, decorin and collagen I. Immunohistochemical analyses demonstrated production of the ECM factors collagen IV, fibrillin 1 and tropoelastin, the cell marker vimentin and the house keeping gene GAPDH. Protein production was noted for GAPDH and SMC specific factor α-actin, and gene expression for vimentin, the ECM components elastin, biglycan and versican and SMC specific factor caldesmon. The quantitative results from Real Time PCR show a lower production of the ECM components compared to native blood vessels, and the expression and protein production of SMC specific factors verify their presence. Although the differences in gene expression between engineered and native vessels are not significant, except for the expression for collagen I, the level of ECM synthesis in these engineered vessels is not enough for the requirements to be used as vascular grafts and can therefore not be used in the clinic.

Synthesis of a biodegradable poly(urethane urea) for tailor-made mechanical properties for vascular scaffold

Basse Asplund[1], Tim Bowden[1], Torbjörn Mathisen[2] and Jöns Hilborn[1]

[1]*Uppsala University, Uppsala, Sweden*
[2]*Radi Medical Systems AB, Uppsala, Sweden*

Background

As a biomedical material, polyurethanes has been used and studied as heart valves and vascular implants due to its hemocompatibility. Polyurethanes are thermoplastic elastomers containing a soft block, acting as a spring, and a hard block holding the material together through physical crosslinking. Today the versatility of polyurethanes and poly(urethane urea)s is controlled by the composition of the soft block and the hard block that holds the material together. Here we present a poly(urethane urea) with a hard block deriving only from diisocyanate, increasing the hydrogen bond capacity. Altering the degree of polymerisation (DP) of the hard block will vary the mechanical and thermal properties. This versatile polymer can easily create vessel like tubes by dip coating a Teflon bar in a polymer solution.

Methods

As the soft block was a two armed poly(ε-caprolactone) (PCL) used and synthesised through ring-opening polymerisation. A pre-polymer was synthesised by adding the dissolved PCL to excess of methyl 2,6-diisocyantohexanoate (LDI). By adding water in a continuous manner in gas phase, using nitrogen as a carrier, the excess of LDI couple via urea linkages. Poly(urethane urea)s was synthesised at a LDI-excess of 6:1, 10:1, 12:1 and 15:1. The synthesis was also performed with other common diisocyanates (HDI, HMDI, TDI and MDI).

Results

Analysis showed that the synthesis was successful. Degree of polymerisation of the hard block was confirmed with NMR and showed a DP(LDI) of 4.8, 8.0, 9.8 and 11.6. Mechanical measurements showed an increase in Young's modulus from 316MPa to 450MPa when DP(LDI) was increased from 4.8 to 11.6, while melting temperature decreased with increasing DP going from 47°C to 39°C.

Comments and conclusions

A new category of poly(urethane urea)s has been developed which creates a new possibility for tailor-made properties. Here we have presented a complementary way of creating a material with mechanical properties similar to blood vessels. Now the properties of poly(urethane urea)s can be varied by altering the soft block but also through the DP of the hard block, the term poly(urethane urea) has been expanded.

Manufacturing and characterisation of bacterial cellulose tubes using two different fermentation techniques

Aase Bodin[a,b], Henrik Bäckdahl[a,c], Lena Gustafsson[b], Bo Risberg[c], Paul Gatenholm[a,].*

a Biopolymer Technology, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Göteborg, Sweden
b Molecular Biotechnology, Department of Chemistry and Bioscinece, Chalmers University of Technology, SE-412 96 Göteborg, Sweden
c Wallenberg Laboratory for Cardiovascular Research, Vascular Engineering Centre, Institution of Surgery, Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden

Cellulose is a natural polymer and has been found to be attractive as a biomaterial because of its stability within a wide range of temperatures and pH levels as well as its good mechanical properties, hydroexpansivity and biocompatibility. Cellulose (β-1→4-glucan) is biosynthesised in vast amounts as structural material in the walls of plants but can be produced as an exopolysaccharide, i.e. Bacterial Cellulose (BC), by *Acetobacter xylinum*. Compared to plant-derived cellulose bacterial cellulose is a hydrogel like material of non-aggregated micro fibrils with high purity [1].

The cellulose can be moulded into desirable shapes for a given application, giving a three dimensional network of micro fibrils [2]. On the micro level, the morphology of the material can be changed in several ways e.g. shaking velocities, oxygen tension, pressure, addition of dyes etc. Our studies have shown that the BC hydrogel has a more dense structure at the liquid/oxygen interface, which might be an advantage for construction of cardiovascular scaffolds. We have evaluated two different fermentation techniques for the manufacturing of bacterial cellulose tubes, using a modified rotating disc reactor. The support for scaffold production was either solid or oxygen permeable.

The study aims to compare the two fermentation techniques and the tubes obtained with regard to carbon consumption and cell proliferation versus yield, porosity, morphology and mechanical properties. The fermentation parameters such as oxygen rate, rotation speed and fermentation time will be optimized to suit the requirements of a scaffold for tissue engineered blood vessels.

Acknowledgements
This work was founded by Vinnova, Swedish Agency for Innovation Systems, SCA hygiene products and EASTMAN chemicals.

References

[1] A. Svensson, E. Nicklasson, T. Harrah, B. Panilaitis, D.L. Kaplan, M. Brittberg, P. Gatenholm. *Biomaterials*. **26** (2005), p. 419-431.

[2] D.G White, R.M. JR Brown, In Schuerch C (ed.), Cellulose and Wood – Chemistry and Technology. New York: Wiley., 1989, p. 573-590.

Decellularised porcine ureter is a biochemically sound and compliant scaffold for tissue engineering of a novel small calibre cardiovascular graft

Yow K H◊, Korossis S*, Ingram J*, Fisher J*, Ingham E*, Homer-Vanniasinkam S♦.*

**Institute of Biomedical Engineering, University of Leeds, ◊Academic Department of Surgery, York District Hospital, ♦Vascular Surgical Unit, Leeds General Infirmary, United Kingdom.*

Background
Current prosthetic alternatives to autologous vascular conduits perform poorly in small calibre anastomosis(under 6 mm) possibly due to compliance mismatch.From evidence, biological extracellular matrices(ECM) have favourable biomechanical and recellularisation properties while obviating immunologic complications.The aim of this study was to investigate a biochemically sound, durable and compliance-matched small diameter vascular substitute using decellularised porcine ureter.

Methods
Porcine ureter from 35 kg animals was decellularised using Tris buffer, 0.1% SDS and nucleases.Controls were untreated femoral artery(FA) and ureter(FURE). Hydroxyproline(HPA) and glycosaminoglycans(GAG) assays assessed the effects of decellularisation on collagen content and ECM respectively.Biomechanical strength was determined by uniaxial tensile testing to failure. Compliance was determined by pressurisation of standardised segments at 20 mm Hg increments from 0 to 300 mm Hg.External diameter changes were measured using 4.0 MPx digital photography and Image Proplus analysis.

Results
Decellularisation was confirmed by H&E histology.There was no disruption in the histioarchitecture post decellularisation while GAG and HPA levels were increased.There was no significant difference in mean ultimate tensile strength(UTS) between acellular scaffold(6.02 ± 0.82 Mpa) and untreated ureter(DURE : 7.0 ± 0.83 Mpa).UTS for DURE was significantly higher than FA(2.28 ± 0.31; $p < 0.05$; ANOVA).

Although FA was more compliant than acellular ureter between 0-60 mm Hg,(0.36±0.08 vs 0.15±0.07% mm Hg^{-1}),at higher pressures there was no difference in compliance(FA: 0.023 ± 0.006; decellularised ureter(DURE):0.037 ± 0.01,$p> 0.05$,ANOVA,n=6).

	HPA (μg/mgTDW)	GAG
FURE	50.37±4.85	27.70±14.6
DURE	51.63±7.9($p>0.05$)	39.89±14.84($p<0.05$,ANOVA)

Conclusions
The acellular scaffold was stronger than FA with matching compliance at physiological pressures.Decellularisation did not diminish the essential biochemistry of the scaffold.Decellularised porcine ureter is hence a potentially suitable biomaterial for vascular tissue engineering.

Polyurethane vascular prosthesis to promote endothelialization from circulating endothelial progenitor cells

Nasser Alobaid, Henryk Salacinski, Kevin Sales, Bala Ramesh, George Hamilton and Alexander Seifalian

Biomaterials & Tissue Engineering Centre (BTEC), University Dept of Surgery, Royal Free and University College Medical School, University College London, London NW3 2PF, UK
Vascular Unit, Dept of Surgery, Royal Free Hampstead NHS Trust Hospital, London NW3 2QG, UK

BACKGROUND:
We hypothesize that endothelial progenitor cells (EPCs) which circulate in the peripheral blood can contribute to endothelialization of vascular grafts. We sought to construct a polymeric scaffold that facilitates endothelialization by providing sites for EPCs adhesion and differentiation. This was done by incorporating Arg-Gly-Asp (RGD) with poly (carbonate-silsesquioxane-urea) urethane (PSU) which we have already shown to be thromboresistant.

METHODS:
We isolated circulating EPCs from volunteers by density-gradient centrifugation. The phenotype of EPCs population was assessed by FACS analysis and RT-PCR for EPCs markers. EPCs were subsequently plated on dishes coated with RGD-PSU, PSU and uncoated dishes and cultured in the presence of vascular endothelial growth factor (VEGF). Cell growth and adhesion were quantified using alamar blue redox assay (ABRA) seven days after plating. We also measured the number of EPCs colonies at day 7. Confirmation of endothelial-cell lineage was performed by immunostaining.

RESULTS:
An average of 1.5×10^7 cells from 20 ml of venous blood was obtained. These cells expressed CD34 as determined by FACS analysis. To further characterize the harvested cells, mRNA transcripts for CD34, CD31, CD133, FlK-1/KDR were detected by RT-PCR; GAPDH was used as quantitative control. Endothelial cells colony forming unit comprising cluster of round cells centrally and sprouts of spindle –shaped cells at the periphery began to appear between day 5 and day 7. EPCs colonies were confirmed by immunostaining with the use of antibodies directed against ecNOS, vWF, and PECAM-1. There were significantly greater numbers of EPCs colonies which appeared on RGD-PSU as compared to PSU and the uncoated dishes. Cells were confirmed to be metabolizing by ABRA.

CONCLUSIONS:
We have developed a vascular graft material that has been demonstrated, *in vitro*, to promote endothelialization from circulating EPCs which suggests potential therapeutic applications.

In vivo biocompatibility of bacterial cellulose

Gisela Helenius[1], Henrik Bäckdahl[1,2], Aase Bodin[2], Ulf Nannmark[3], Paul Gatenholm[2], & Bo Risberg[1]

[1)]Wallenberg Laboratory for Cardiovascular Research, Vascular Engineering Centre, Institution of Surgery, Sahlgrenska Academy, Göteborg University, Göteborg, Sweden
[2)]Department of Polymer Technology, Chalmers University of Technology, Göteborg, Sweden
[3)]Electron Microscopy Unit, Institution of Anatomy and Cell Biology, Sahlgrenska Academy, Göteborg University, Göteborg, Sweden

A scaffold for tissue engineered blood vessels is supposed to mimic the native extracellular matrix and guide the cells in the process of tissue formation. A possibility to modify the surface properties, porosity, and the ability to design and shape three dimensional structures is also desirable. The most obvious requirement is however the biocompatibility. The definition of biocompatibility, according to Williams in 1987, is: "the ability of a material to perform with an appropriate host response in a specific application". Bacterial cellulose (BC) is a material that not has been used widely in tissue engineering before. BC is synthesised extracellularly into nano-sized fibrils by the bacteria *Acetobacter Xylinum* and has many unique properties that can be useful. It is known from before that cellulose-based materials induce only negligible foreign body and inflammatory responses and they are considered as biocompatible. In the development of tissue engineered blood vessels with a bacterial cellulose scaffold it is however necessary to evaluate the *in vivo* biocompatibility of the biomaterial.

Pieces of BC were implanted subcutaneously in female Wistar rats. After 1, 4 and 12 weeks, the BC pieces were removed, fixed and further processed for electron microscopy, histological analyses and immunohistochemistry. To determine the biocompatibility of BC following parameters were analysed: cell ingrowth, inflammation, foreign body reaction, tissue integration and angiogenesis around the implant.

After 1 week, cells had migrated into the material. From the compact side, only a few cells, with small round nuclei, sporadically had penetrated the BC. At the porous side, cells were also aligned at the surface. From the porous side, a united front line of cells with small round nuclei had migrated in approximately xx μm. Cellular ingrowth was very much dependent of the material density and declined when the BC got denser.

Macroscopically, no signs of inflammation were visible. Staining with antibodies against macrophages turned out to be negative. Neutrofils with three lobular nuclei were also absent. Neither detectable capsule formations nor giant cells were present around the implants, instead, the rat connective tissue, very nicely integrated with the BC. Newly formed blood vessels were seen around and growing into the implant.

BC is a promising biomaterial to be used as scaffold for tissue engineered blood vessels.

Electrospinning of collagen and elastin: a novel scaffold for cardiac tissue engineering

Sepideh Hagvall[1,2], Yuhuan Xu[1], Ben Wu[1], Bo Risberg[2], Ramin Beygui[1]

[1] Department of Bioengineering, University of California, Los Angeles, California
[2] Vascular engineering centre, Department of Surgery, Sahlgrenska University Hospital, Göteborg, Sweden

Background

Loss of cardiac myocytes accounts for a decrease in myocardial function which can lead to total organ failure. In end stage heart failure, heart transplantation remains the last treatment option. Unfortunately, heart transplantation is limited due to an inadequate supply with donor organs. There is an obvious need to improve traditional treatment and develop new strategies. An alternative approach is the design of tissue engineered cardiac muscle constructs *in vitro* for later implantation *in vivo*. To date, many investigators have developed materials such as collagen mixtures, PLA, and PGA for use as scaffold construction with improved, yet unacceptable, clinical results.

Aim

The present study aims to use electrospinning technique to construct a novel scaffold, made of collagen and elastin, in order to engineer beating cardiac patches for transplantation.

Materials and methods

A mix of collagen and elastin (2:1), dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol, was loaded into a syringe which was attached to a 25 gauge needle. In a high electric field, the scaffold was electrospun as a flat mat on the target. The electrospun scaffold were then cross-linked in glutaraldehyde vapors for 4h at RT. Neonatal rat cardiomyocytes were seeded (0.5-1 x 10^6 cells/cm^2) on the electrospun scaffold for 5 days at 37ºC and 5% CO_2.

Preliminary results

In this ongoing study, collagen and elastin have been electrospun to produce nano- to micro-diameter fibers. So far, we have observed extensive cell attachment into the electrospun collagen-elastin scaffold, also cell-to-cell contact and communication through gap junctions, resulting in simultaneous beating after three days in culture.

Bacterial cellulose as a scaffold for endothelial cells in tissue engineered vascular grafts

Helen Fink, Gisela Helenius, Paul Gatenholm, Bo Risberg

Bacterial cellulose (BC) is a biomaterial that can be used as a graft or as a scaffold in a tissue-engineered blood vessel (TEBV). It is important for the success of a tissue engineered vascular graft, that the endothelial cells can adhere to the biomaterial and form a confluent monolayer. Therefore, the aim of this study is to investigate the adhesion of endothelial cells to BC with both qualitative and quantitative methods.

Human saphenous vein endothelial cells (HSVEC) were seeded onto BC, compressed BC, air-dried BC and BC treated with acetone. Cell-culture polystyrene and Matrigel™ were used as reference materials. The adhesion was quantitatively measured by the AlamarBlue™-assay, counting of nuclei and counting of non-adherent cells. The morphology of the HSVEC was qualitatively studied by staining the cells against PECAM-1 (CD31), f-actin and DNA.

In the AlamarBlue-assay a difference could be observed between the various forms of BC as well as between the reference materials and the BC. Air-dried and acetone treated BC displayed the highest adhesion rate but it was not as high as compared with polystyrene. The morphological study shows that HSVECs express PECAM-1 in a normal manner and form a normal endothelial cell structure.

In conclusion, these results indicate that the adhesion of HSVEC to BC is comparable with Matrigel and suggests that the density of the fibre network could alter the adhesion of HSVEC to BC.
BC has proven to have a good potential for pre-seededing with endothelial cells and could therefore be used as a scaffold for TEBV.

INFLUENCE OF FLUID SHEAR FORCES ON HUMAN CORONARY ARTERY SMOOTH MUSCLE CELLS

J Ghosh, MO Murphy, MG Walker, CM Kielty

UK Centre for Tissue Engineering, University of Manchester, Manchester. UK.

BACKGROUND:
The aim of this study was to examine the influence of *in vitro* shear stress on human coronary artery smooth muscle cells (HCASMC), focussing specifically on extracellular matrix (ECM) synthesis, cell proliferation, and expression and organisation of cytoskeletal proteins. This study has also addressed the role of Transforming Growth Factor (TGF) β_1 in mediating HCASMC phenotype alterations under these conditions.

METHODS:
HCASMC cultured under physiological conditions were subjected to degrees of fluid shear stress within a bioreactor. Expression of extracellular matrix molecules and cytoskeletal components were determined by RT-PCR and immunofluorescence. Cell orientation and organisation of α-actin filaments were examined microscopically. The proportion of cells undergoing mitosis was identified by detection of Ki67 nuclear protein. RT-PCR and ELISA determined expression and release of the cytokine TGFβ1 and latent TGF-beta binding protein (LTBP) 1 and 3. The effect of shear stress on extracellular matrix synthesis, proliferation, cytoskeletal expression and organisation following blockade of TGFβ1 signalling was also assessed.

RESULTS:
Fluid shear stress resulted in rapid SMC cytoskeletal remodeling and dedifferentiation characterised by increased proliferation and ECM synthesis, in particular collagen type I, collagen type VIII, fibrillin 1 and elastin. Concurrent was increased $TGF\beta_1$ mRNA transcription and active $TGF\beta_1$ concentration in the growth media. Furthermore, $TGF\beta_1$ signalling was demonstrated. Blockade of the $TGF\beta_1$ receptor heterodimer abrogated the changes in SMC proliferation and ECM synthesis but not cytoskeletal remodelling.

CONCLUSION:
Upon direct exposure to fluid shear stress equivalent to luminal flow exhibit increased proliferation, dedifferentiation and cytoskeletal reorganisation. This work suggests that two key features in the pathogenesis of myointimal hyperplasia development, increased smooth muscle cell proliferation and extracellular matrix synthesis, is mediated via a TGFβ1 autocrine mechanism.

The effect of shear stress on the expression of coagulation and fibrinolytic factors in both smooth muscle- and endothelial cells in a co-culture model.

G. Helenius, S.H. Hagvall, R. Söderberg & B. Risberg

Vascular Engineering Centre, Department of Surgery, Sahlgrenska University Hospital, Göteborg, Sweden

The blood vessel is constantly subjected to mechanical forces due to the blood flow. The endothelial cells (EC) are recipients of shear stress and through a close cross-talk with the underlying smooth muscle cells (SMC), the vessel regulates important physiological processes. Disturbance in the flow pattern leads to pathological conditions.

The EC response to shear stress has been widely investigated. Although EC and SMC co-exist in the vessel wall, less is known about the effect of shear stress when cultured together. Therefore, it was hypothesized that, in a co-culture, both EC and SMC can respond to shear stress which leads to a change in the expression of factors in the coagulation- and fibrinolytic systems.

Human saphenous vein endothelial cells (HSVEC) were cultured direct on top of human saphenous vein smooth muscle cells (HSVSMC). The co-culture was exposed to physiological levels (12 dyn/cm^2) of laminar shear stress for 4 and 24 hours. After conclusion of the flow experiments, the two cell types were separated using an antibody based technique. Gene expression of tissue factor (TF), thrombomodulin (TM), plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) in both HSVEC and HSVSMC was analyzed with real time RT-PCR. Intracellular protein expression was studied with ELISA.

Significant changes of the studied factors were present in both HSVEC and HSVSMC.

The main findings of the present study were that both HSVEC and HSVSMC responded to shear stress, which lead to an altered gene and protein expression of coagulation and fibrinolytic factors. HSVSMC have the potential to sense shear stress indirectly via the HSVEC through mechanisms that could be either chemical or mechanical. This indicates that SMC, and the interactions between EC and SMC, are more important in the regulation of vascular wall haemostasis than earlier studies have reported. Further studies to elucidate the mechanisms behind both the responses of shear stress and the interactions between EC and SMC are required.

Investigation of the effects of flow on human endothelial cells seeded in cylindrical conduits representing vascular grafts

D.S. Vara[1], G. Punshon[1], K.M. Sales[1], H.J. Salacinski[1], G. Hamilton[1,2], A. M. Seifalian[1]
[1]Biomaterials & Tissue Engineering Centre, Academic Division of Surgical and Interventional Sciences, University College London, Hampstead, London, UK and [2]Royal Free Hampstead NHS Trust Hospital, London, U.K.

Introduction

Mechanical forces created by blood shear stress have been known to play a fundamental role in the vessel wall. Vascular cells respond to these stimuli by regulating the expression of many genes involved in cell proliferation, differentiation and the subsequent maintenance of vascular tone, thrombosis, cell-matrix and cell-cell adhesion and inflammation. Shear stress preconditioning of EC seeded grafts has made a major impact in vascular tissue engineering and has been shown to promote EC retention and differentiation on the inner graft surface. The aim of this study was to assess the effects of flow on the gene expression of human endothelial cells seeded in a cylindrical model, representing a vascular graft.

Methods

Human umbilical vein endothelial cells (HUVEC) were isolated and cultured in flasks to confluence. $1x10^6$ cells were seeded onto 5mm diameter glass tubes (5cm length) for 24 hrs and an Alamar Blue™ assay performed to assess cell viability. We have developed and validated a flow system able to simulate in vitro the pulsatility and flow waveform. We used this model to determine EC viability and gene expression on seeded glass tubes exposed to physiological shear stress. The model consisted of a variable-speed pump, tubing and reservoir, flow waveform conditioner, oxygenator, outflow resistance, tubular flow probe, Transonic Flowmeter and catheter transducer. The circuit was filled with media. A flow rate of 200 ± 15 ml/min was applied for 1 and 4 hrs. Static samples were placed in fresh medium and incubated at 37°C for 1 and 4 hrs. Alamar Blue™ assay was performed post flow on static and flow samples. HUVEC were harvested using trypsin after exposure to shear stress. RNA was extracted using a Qiagen RNeasy™ kit. Polymerase chain reaction (Figure 1.) for the genes of the house keeping protein Glyseraldehyde-3-phosphate dehydrogenase (GAPDH); the extracellular matrix proteins collagen I (COL-1); tumor growth factor beta (TGF-β); and the adhesion molecule platelet endothelial cell adhesion molecule-1 (PECAM-1).

Results

Alamar Blue™ results post seeding showed viable cells present on all seeded tubes. After application of flow, there was no significant difference in cell viability between the static and post flow samples. HUVEC exposed to flow post-1hr showed increase in expression of TGF-B and COL-I compared to static samples. There was no change in PECAM expression between the static and post-1hr flow samples. Exposing HUVEC to 4hrs of shear stress showed a substantial increase in TGF-B, COL-I and PECAM expression compared to static samples (Figure 2.).

Discussion

The Alamar Blue™ results obtained clearly indicate that the cell number has not changed significantly and that the cells have, and/or are in the process of, altering their cognitive physiology in order to adapt to the shear stress applied in the vascular graft model.

Altered gene expression demonstrated that that the cells are adapting to a flow environment as experienced in cylindrical conduits. TGF-β is a growth factor that causes cellular differentiation and is key in the development in extra cellular matrix such as COL- I. These results are consistent with those observed by other researchers. The up regulation of the adhesion molecule PECAM-1 at four hours further confirms our hypothesis. In conclusion we have developed and validated successfully a model of vascular grafts that is of use to biomaterials and tissue engineering research focusing on the role of gene expression in their clinical patency.

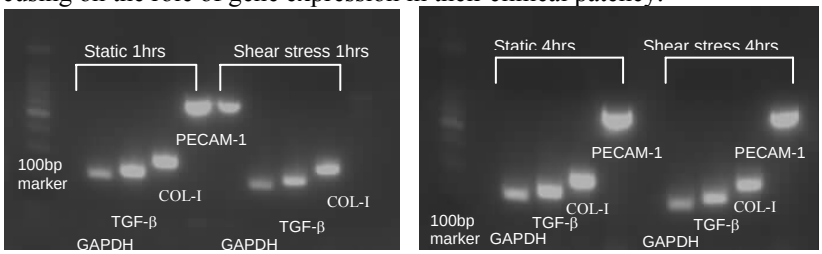

Figure 1. 2% agarose gel representing gene expression in static and shear stress samples post a) 1hr shear stress and b) 4 hrs of shear stress

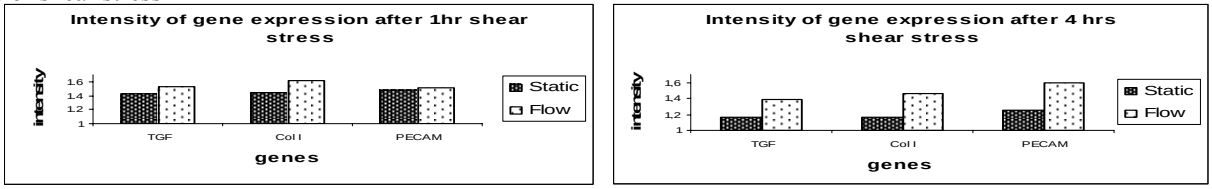

Figure 2. Intensity of gene expression in static and shear stress samples post a) 1hr shear stress and b) 4hr shear stress

The effects of physiological and pathological stretch on vascular smooth muscle cells

AT Halka[ab], J Ghosh[ab], MO Murphy[ab], N Khwaja[a], NJ Turner[b], CM Kielty[b], MG Walker[ab]

[a]*Department of Vascular Surgery, Manchester Royal Infirmary, Manchester, UK*
[b]*UK Centre for Tissue Engineering, University of Manchester, UK*

Background

Cardiovascular disease causes more deaths in the United Kingdom than all types of cancer combined. Numerous preventative measures including anti-smoking campaigns are of limited value. Drug therapy slows disease progression but more invasive methods are required to treat obstructed blood vessels. This can take the form of surgery that includes bypass grafting and/or radiological intervention. The results of these interventions may be disappointing in the acute as well the chronic phase with thrombosis, obstruction and infection being particular problems. The purpose of the emerging science of vascular tissue engineering is to synthesise artificial conduits that bear a closer resemblance to the natural vasculature. This characteristic could then be harnessed to reduce the risks of graft failure and thus improve outcomes.

Methods

Human coronary artery smooth muscle cells from a nineteen year old male were used at varying passages. They were cultured on pre-coated silicone membranes which were then subjected to physiological and pathological stretch using a computerised system produced by Flexcell International (Hillsborough, North Carolina, USA). Immunofluorescence and polymerase chain reaction techniques were employed to determine differences between control and stretched samples.

Results

Immunofluorescence demonstrated variable smooth muscle alpha actin and F-actin expression. DNA was obtained from cells acting as controls and those exposed to stretch by employing the polymerase chain reaction and utilising alpha actin, myosin and calponin primers. The expression of these aforementioned markers of contractility within the smooth muscle cell was influenced by the degree and the period of stretching. The coating on which the cells were cultured also influenced these results.

Comments and conclusions

Vascular smooth muscle cells are the major determinants of blood vessel tone. They also produce extracellular matrix and provide support for other components of the vessel wall. Determining and understanding the way vascular smooth muscle cells transduce the mechanical force of stretch is crucial if the vasculature is to be engineered successfully.

Umbilical cord blood derived endothelial progenitor cells for vascular tissue engineering : an in vitro study in response to physiological shear stress

X. Bérard, M. Rémy-Zolghadri, Ch. Bourget, N. Turner[1], M. Renard[2], R. Bareille, L. Bordenave

INSERM U577 "Biomaterials and Tissue Repair"; Université Victor Segalen, Bordeaux, France
[1]UK Centre for Tissue Engineering, University of Manchester, Manchester, UK
[2]Centre d'Innovations Technologiques, CHU de Bordeaux, Place A. RabaLéon, Bordeaux, France

Introduction

The aims of the present study were to check whether endothelial progenitor cells (EPCs) derived from cord blood could be an effective source for vascular tissue engineering and to investigate their *in vitro* behaviour on a prosthetic device in response to shear stress (SS) at the mRNA level.

Materials and methods

CD34+ mononuclear cells were isolated from cord blood by a magnetic beads separation and cultured under endothelial conditions. EC characterization was assessed by immunocytochemistry for CD31 and von Willebrand factor (vWF), flow cytometry analysis for VE-cadherin and western blotting for KDR. Cells were used for adhesion and proliferation assays on 6 mm ID collagen coated Dacron grafts (Laboratoire Perouse, France). The resistance to 6 h-SS at 16 dyn/cm² of EPCs was evaluated by histology and confocal laser scanning microscopy (CLSM). After 4h of SS exposure, relative quantification of mRNA levels for MMP1, VE-cadherin and KDR was performed.

Results

CD34+ cells differentiate withan endothelial phenotype : derived ECs are positive for CD31, vWF, VE-cadherin and express KDR. EPCs adhere to vascular grafts in serum-free conditions. EPC proliferation showed a complete cell coverage visualized by histology (positive staining for CD31 and CD34) and CLSM. CLSM demonstrates that SS prevented cell detachment from grafts compared with controls. Four hours of SS at 16 dyn/cm² significantly increased the expression of 3 genes: 1.53 ± 0.38, 2.2 ± 0.76 and 2.1 ± 0.65 (mean ± SD, n= 3) for sheared/static ratios for MMP1, VE-cadherin and KDR, respectively.

Discussion

Umbilical cord derived progenitor ECs are able to functionalize vascular grafts, to resist to SS and to be modulated by SS in terms of gene expression.

ACKNOWLEDGEMENTS

Laboratoires Perouse, Fondation pour la Recherche Médicale, Centre d'Innovations Technologiques (CHU de Bordeaux).

Strain-based optimisation of tissue-engineered small diameter blood vessels

M. Stekelenburg[a], M.C.M. Rutten[a], F.P.T. Baaijens[a], L.H.E.H. Snoeckx[b]

[a]Department of Materials Technology, Department of Biomedical Engineering, Eindhoven University of Technology
[b]Department of Physiology, Maastricht University

Creating a small-diameter blood vessel substitute is one of the goals of tissue engineering. The object of this study is to find the optimal mechanical conditioning protocol to optimize the development of tissue engineered blood vessel constructs. To reach this goal, the effect of axial and circumferential strain, the main physiological mechanical stimuli in a coronary artery, are investigated separately as well as in combination with each other.
Two experimental set-ups were developed. In the first set-up, constructs are subjected to a circumferential strain by applying a pulsatile flow to the tubes. In the second device the constructs are axially strained in combination with a flow. The two set-ups can be combined to subject the constructs to axial and circumferial strain simultaneously.

The scaffold material that is used is P4HB coated PGA in combination with a fibrin gel. This combination already proved its potential in tissue engineered heart valves. Human vena saphena cells were seeded in tubular constructs and cultured under mechanical conditions (5% axial or circumferential strain) for 4 weeks.
Histological examination of the cultured constructs showed a homogeneous tissue formation throughout the thickness of the wall. Masson trichrome staining confirmed the presence of collagen. In case of the circumferentially strained tubes, cells are aligned circumferentially at the inside of the tube, in the outer layers cells are more axially orientated. The orientation in the axially strained constructs is more uniform. DNA, GAG and hydroxyproline content were measured. Cultured constructs will be tested mechanically in circumferential and axial direction. Preliminary results show Young's moduli in the range of 2MPa.
The strain in axial or circumferential direction during culture will be related to the mechanical properties of the constructs in these directions. In this way we expect to guide the mechanical properties by providing the appropriate strains in both directions.

The Use of Recombinant Type VIII Collagen as an Attachment Factor For Endothelial cells cultured under static and shear stress flow

N. J. Turner, C.M. Kielty, M.G. Walker, A. E. Canfield

UK Centre for Tissue Engineering, University of Manchester, Manchester, M13 9PT, UK.

Background

Endothelialisation has been identified as a key requirement for the success of any synthetic or tissue engineered vascular graft. However, implanted vascular grafts do not naturally develop a complete endothelial layer. The aim of this study was to determine whether recombinant type VIII collagen chains could promote endothelial cell attachment and increase cell retention under shear stress flow.

Methods

The α2(VIII) chain of type VIII collagen was cloned, his-tagged and used to produce recombinant collagen homotrimers using an in vitro expression system. Confirmation of a stable triple-helix was obtained using pepsin and collagenase digests.

Samples of purified α2(VIII) collagen (0 to 1000 ng/ml) were used as substrata for endothelial cell attachment, with endothelial cell attachment assessed after 30 minutes using a crystal violet dye elution method. Endothelial cell retention to α2(VIII) collagen substrata under mean shear stress flow of up to 100 dynes/cm^2 was also assessed using a parallel plate flow system. To investigate the mechanism of endothelial cell attachment blocking assays were performed using RGD peptides and antibodies against integrin subunits α2, α3, α5 and β1.

Results

Endothelial cells attached to recombinant α2(VIII) collagen in a dose dependent manner with a concentration of 1000ng/ml giving comparable levels of attachment to fibronectin at a concentration of 10μg/ml. Compared to fibronectin substrata under shear stress flow, α2(VIII) collagen promoted significantly higher levels of cell retention at shear rates of between 30 and 60 dynes/cm^2. Blocking assays have identified that cell attachment to α2(VIII) collagen is RGD dependent and possibly mediated by the α3 and β1 integrins.

Conclusion

Recombinant α2(VIII) collagen homotrimers have significant potential for use in tissue engineering as an attachment factor for endothelial cells.

Cyclic strain modulation of gene and protein expression in human mesenchymal stem cells (hMSCs) compared with human vascular smooth muscle cells (SMCs)

Sepideh H.Hagvall[a], Maricris Esguerra[a], Tommi Tallheden[b], Erik Ulfhammer[c], Sverker Jern[c] and Bo Risberg[a]

[a] Vascular engineering centre, Department of Surgery, [b] Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska University Hospital, Göteborg, Sweden
[c] Clinical Experimental Research Laboratory, Sahlgrenska University Hospital/Östra, Göteborg, Sweden

Background
Tissue engineering allows the design of functionally active autologous cells, which have the potential to minimize foreign body-host reactions. Recent studies have demonstrated that adult hMSCs have the potential to differentiate into characteristic vascular cells.

Methods
Human SMCs and hMSCs were cultured on plates with flexible silicone rubber bottoms and subjected to equibiaxial cyclic strain of 14% for 4h and 24h.

Results
The results indicate time-dependent changes in gene and protein expression of SMCs specific markers, cytoskeleton and extracellular matrix-components in both SMCs and hMSCs.
In SMCs, the gene expression of α-actin and lysyloxidase was significantly down-regulated and the gene expression of vimentin, decorin and versican was significantly up-regulated by cyclic strain. Significantly up-regulation of caldesmon gene expression after 4h and down regulation after 24h cyclic strain was observed in SMCs. In SMCs the protein production of α-actin, calponin and decorin was higher after 24h and the protein production of collagen I was higher after 4h cyclic strain.
In hMSCs, the gene expression of α-actin, caldesmon, versican, biglycan, fibronectin and collagen I was significantly down-regulated and the gene expression of vimentin and lysyloxidase was significantly upregulated by cyclic strain. Higher protein production of α-actin, calponin and collagen I was observed by cyclic strain in hMSCs.

Conclusions
In this study we have demonstrated that hMSCs are capable of responding to mechanical stress and they hold great potential as a cell source of autologous vascular cells.

Real time measuring of blood coagulation density and immune complement activation on polymer and metal surfaces with QCM-D (Quartz Crystal Microbalance with Dissipation monitoring) technique

Marcus Andersson[1], Jonas Andersson[2], Anders Sellborn[1,3], Mattias Berglin[1], Bo Nilsson[2] and Hans Elwing[1]

[1]Department of Cell and Molecular Biology/Interface Biophysics Lundberg Laboratory, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden.
[2]Department of Oncology, Radiology and Clinical Immunology, Section of Clinical Immunology, Rudbeck Laboratory C5, Uppsala University Hospital, SE-751 85 Uppsala Sweden
[3]Vascular Engineering Centre, Department of Surgery, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden

A recently developed variant of quartz crystal microbalance (QCM) called QCM-with dissipation monitoring (QCM-D) allows simultaneous and simple measurements of changes in adsorbed mass as well as the viscoelastic property (D-factor) of deposited protein layers on the sensor surface. We have taken the QCM-D technology a step further and demonstrated its advantages in the study of protein assembly as a consequence of surface induced immune complement activation, or contact activated blood coagulation. In the present study we have continued our QCM-D investigations of surface assembly of fibrin clot formation and complement activation and incubated differently modified quartz sensor surfaces in blood plasma and sera. Polymer surfaces used were spin-coated polyethylene, poly(ethylene terephtalate), poly(methylmetacrylate) and poly(dimethylsiloxane). Also used were sputtered titanium and heparin grafted surfaces. In this investigation we found that we could describe the surface induced coagulation with four independent parameters: 1) Time of onset of coagulation, 2) fibrin deposition rate, 3) total frequency shift at stable plateau, and 4) fibrin clot density. The most important finding was that the blood plasma clot density can be assessed with the use of D determinations and that the clot density varied significantly with the chemical composition of the surface. However, the D-factor did not give any new analytical information about the possible complement activation mechanisms. Nevertheless, the QCM-D was found to be a reliable tool for the analysis of surface induced complement activation.
We also compared the QCM-D technique with traditional enzyme immuno assay (EIA) measurements of soluble products from the surface activation of the complement and coagulation systems. We found that the results from EIA and QCM-D measurements corresponded well for the complement activation but not for the coagulation, probably due to the biological complexity of the coagulation system.

Shear stress transduction in endothelial cells lining a tissue-engineered blood vessel

Rémy-Zolghadri M., Oligny♦ JF., Germain♦ L., Auger♦ FA*

♦ *Laboratoire d'Organogénèse Expérimentale – Saint-Sacrement Hospital – Laval University – Québec - Canada*
* *INSERM U-577 – Victor Ségalen University – Bordeaux - France*

We produced in 1998' a tissue-engineered blood vessel (TEBV) with cultured human cells. This TEBV displays the histology of a native vessel and supports mechanical strain. The functional characteristics of cells into the TEBV are promising since the media's constractility and the intima's anti-thrombogenicity in static condition are restored. The behaviour of endothelial cells [EC] under shear stress [SS] is now investigated to ensure cell responses in conditions mimicking arterial flow.

First, EC lining the TEBV internal layer were subjected to increasing shear stress values (until 15 dyns/cm^2) during 1 h to ensure their adherence to the underlying matrix. Subsequently, the cells were exposed to 15 dyns/cm^2 for 10, 30, 60 and 120 minutes before analyzing their responses. Since the SS is integrated by cells via the MAP Kinase pathways, P42/44 and P38 activation were analysed by western blots of protein phosphorylation under SS compared to results obtained in static conditions.

The cells remained adherent whatever the SS value and elongated when a progressive SS was applied. When the value of SS directly applied (i.e. 15 dyns/cm^2) was comparable to arterial SS, cells respond by the activation of P42/44 and P38, as attested by the ratios of phosphorylated protein to total protein. These values always remained superior for cells under SS compared to that of the control over time (for up to 2h) suggesting a sustained activation of cells.

In conclusion, we confirmed the functional status of EC in our TEBV. They are able of an adaptative response under SS probably via activation of MAPKs. Further experiments will be designed to assess under SS the EC properties previously evaluated in static conditions.