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Dynamics of Extracellular Matrix Production and Turnover in Tissue Engineered Cardiovascular Structures

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Abstract Appropriate matrix formation, turnover and remodeling in tissue-engineered small diameter vascular conduits are crucial requirements for their long-term patency and function. This complex process requires the deposition and accumulation of extracellular matrix molecules as well as the remodeling of this extracellular matrix (ECM) by matrix metalloproteinases (MMPs) and their endogenous inhibitors (TIMPs). In this study, we have investigated the dynamics of ECM production and the activity of MMPs and TIMPs in long-term tissue-engineered vascular conduits using quantitative ECM analysis, substrate gel electrophoresis, radiometric enzyme assays and Western blot analyses. Over a time period of 169 days in vivo, levels of elastin and proteoglycans/glycosaminoglycans in tissue-engineered constructs came to approximate those of their native tissue counter parts. The kinetics of collagen deposition and remodeling, however, apparently require a much longer time period. Through the use of substrate gel electrophoresis, proteolytic bands whose molecular weight was consistent with their identification as the active form of MMP-2 (≈64–66 kDa) were detected in all native and tissue-engineered samples. Additional proteolytic bands migrating at ≈72 kDa representing the latent form of MMP-2 were detected in tissue-engineered samples at time points from 5 throughout 55 days. Radiometric assays of MMP-1 activity demonstrated no significant differences between the native and tissue-engineered samples. This study determines the dynamics of ECM production and turnover in a long-term tissue-engineered vascular tissue and highlights the importance of ECM remodeling in the development of successful tissue-engineered vascular structures. J. Cell. Biochem. 81:220–228, 2001. © 2001 Wiley-Liss, Inc.

Key words: extracellular matrix; remodeling; tissue engineering; blood vessels

Extracellular matrix remodeling, as observed during development [Liu et al., 1999; Vaillant et al., 1999], wound healing [Neely et al., 1999; Shapiro et al., 1992] and after vascular injury [Meng et al., 1999; Smith et al., 1999], is a complex process which requires a tight regulation of regenerative and degenerative processes [Buckley-Sturrock et al., 1989]. It is well appreciated that the dysregulation of these processes in the form of excessive matrix formation can result in fibrotic disease [Karim et al., 1991; Peters et al., 1997]. This is particularly important in mechanically active tissues such as blood vessels where fibrosis can alter the biomechanical characteristics leading to impaired function as a result of reduced contractility and distensibility as well as luminal narrowing and occlusion [Liu et al., 1989; Granke et al., 1993].

Extracellular matrix (ECM) remodeling has become a major area of interest in the emerging field of tissue engineering. The multidisciplinary approach of tissue engineering utilizes biodegradable polymer scaffolds and autologous cellular components in an attempt to generate viable arterial grafts with the ability to function, grow, repair and remodel [Langer and Vacanti, 1993]. In order to obtain tissue with the ability to withstand mechanical stresses, such as the impact of blood pressure, tightly

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regulated matrix production and deposition is necessary. Critical for the long-term function of these tissues, however, is appropriate matrix remodeling with the ultimate goal of the establishment and maintenance of a delicate equilibrium between regenerative and degenerative mechanisms. Despite encouraging early results, the in vivo patency of tissue-engineered blood vessels (internal diameter (i.d. >3 mm) has been limited by subsequent narrowing and occlusion with a maximal reported graft patency of 28 days. This problem has significantly limited the long-term value of these vessels [Shum-Tim, 1997; Niclason et al., 1999].

These limitations have led us to hypothesize that dysregulated matrix remodeling, at least in part is responsible for such frequent vessel occlusion. The purpose of this study, therefore, is to determine and compare the activity levels of the enzymes and inhibitors that are responsible for extracellular matrix remodeling in order to determine whether the occlusion of tissue-engineered vascular grafts is caused by increased matrix deposition, decreased matrix degradation or both. Given that the activity of the MMP family of proteinases is a key limiting step in ECM degradation, we have focused on this family of enzymes and their endogenous inhibitors. We have used a recently established valved conduit model (18 mm i.d.) to investigate the long-term deposition, degradation and turnover of extracellular matrix for up to 169 days [Stock et al., 2000]. Elucidation of the dynamic process of tissue formation and the regulatory mechanisms of tissue turnover will contribute to our understanding and control of the pathologic matrix accumulation in tissue-engineered small diameter blood vessels, which had become a major challenge in this emerging field.

MATERIALS AND METHODS

Cell Isolation and Culture

Carotid artery segments were harvested from 6-week-old Dover lambs ($n = 9$). After recovery, the animals were housed in an indoor housing facility until graft implantation. Endothelial cells (EC) were obtained using an intraluminal collagenase type A (0.2% in 1% bovine serum albumin, Roche Corp, Indianapolis, IN) instillation technique (20 min at 37°C at 95% O_2 and 5% CO_2) [Jaffe et al., 1973]. EC were cultured in Medium 199 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO), 1% L-glutamine, penicillin, streptomycin (Gibco) and 50 IU/ml heparin (Elkins-Sinn Inc., Cherry Hill, NJ) in gelatin-precoated tissue-culture flasks. The media was changed every 3 days. Identity of EC was confirmed by immunostaining for human van Willbrand factor (rabbit anti-human, DAKO Corp., Carpintera, CA) with cross-reactivity to sheep. The remaining de-endothelialized vessel segments were sliced into 1 mm^2 pieces and cultured in DMEM high glucose (Gibco) supplemented with 10% fetal bovine serum, 1% L-glutamine, penicillin, streptomycin on P100 dishes. After allowing the cells to migrate onto the dishes (5–7 days after plating), the cells (mixed culture of smooth muscle cells and fibroblasts) were serially passaged [Campbell and Campbell, 1993]. Smooth muscle cells were identified by immunostaining with a monoclonal antibody to anti-α smooth muscle actin (mouse ascites, Sigma).

Biodegradable Polymer

The conduit wall was composed of 240 μm non-porous polyhydroxyoctanoate (PHO 3836; TEPHA Inc., Cambridge, MA) surrounded by two non-woven 1 mm thick polyglycolic acid (PGA) felts (Smith and Nephew Inc., Kent, UK). The leaflets consisted of a monolayer of porous PHO (120 μm thickness, pore size 50–150 μm). In order to facilitate cell–polymer attachment the polymer characteristics were altered by a salt-leaching technique [Mooney et al., 1995]. Briefly, sieved sodium chloride crystals ($50 < d < 150$ μm) were evenly distributed into melted PHO resulting in a polymer film with entrapped NaCl particles. After cooling, the salt particles were leached out of the film by immersion in deionized water. The leaflets were sutured into the conduit using 6-0 PDS running sutures. The conduits were immersed for 24 h in 2% polyvinylalcohol to increase hydrophilicity and eventually cold gas sterilized (ethylenoxide).

Cell Seeding

For improved cell–polymer attachment the polymer constructs were precoated with laminin (5 μg/ml, for 24 h, Sigma). Twenty-three days (± 3 days) after the vessel harvest, 17.38×10^6 ($\pm 0.9 \times 10^6$) fibroblasts and smooth muscle cells were seeded onto the construct on four consecutive days. Endothelial cells 14.85×10^6

($\pm 0.25 \times 10^6$) were seeded once on Day 27. The constructs were cultured in DMEM/high glucose supplemented with 10% fetal bovine serum, 1% L-glutamine, penicillin, streptomycin. After one final incubation day the constructs were implanted into the sheep.

In Vivo Implantation

Thirty-one (± 3 days) after the initial harvest, the heart was exposed by a left-sided thoracotomy ($n = 8$). Using cardiopulmonary bypass, the native pulmonary artery (PA) was transected and a segment of the PA as well as all three pulmonary valve leaflets were removed and the constructs (seeded with autologous cell) implanted. No postoperative anticoagulant was administered. One further construct was seeded, but not implanted, and served as a control. The animals were sacrificed after 5, 14, 29, 41, 55, 85, 112, and 169 days. The conduits and the adjacent native PA were explanted and immediately frozen at −80°C. Samples from the conduit were obtained from the inflow region (0.5 cm proximal the leaflets). Tissue from the native PA was at least 1 cm proximal to the suture line in order to eliminate any interference from the surgical procedure. All samples were evaluated for their content of collagen (4-hydroxyproline), elastin, proteoglycan/glycosaminoglycan content and activity of metalloproteinases (MMPs) and tissue-inhibition metalloproteinases (TIMPs). In addition, the intimal thickness of the internal conduit wall was evaluated.

Protein Determination

For 4-hydroxyproline, elastin, proteoglycan/glycosaminoglycan and MMPs as TIMPs assays all samples were normalized according to equivalent protein concentrations. Protein content was determined using a Bio-Rad (Hercules, CA) protein assay kit according to the manufacturer's specifications with bovine serum albumin as the standard.

4-Hydroxyproline Extraction and Determination

Tissue samples were thawed, rinsed in PBS and minced. 4-Hydroxyproline (4-OHP) was extracted according to the method of Bergman and Loxley [1963]. Briefly tissue samples were digested in 6 N HCl overnight at 100–110°C, redissolved in 6 N HCl, decolorized with activated charcoal and, finally, neutralized with and equal volume of 6 N NaOH. Samples were mixed with isopropanol and a 1:4 solution of chloramine T and acetate–citrate buffer and incubated for 4 min at room temperature. After adding a 3:13 solution of *p*-dimethylaminobenzaldehyde in $HClO_4$ (Sigma), the samples were incubated for 25 min at 60°C and cooled. Optical density was read spectrophotometrically at 558 nm. 4-OHP concentration was expressed as micrograms per milligram protein.

Elastin Extraction and Determination

Tissue samples were thawed, rinsed in PBS and sliced. Elastin was extracted with 0.25 N oxalic acid (Sigma) at 95°C for 1 h [Kim et al., 1998]. After rapid cooling and spinning at 3,000 rpm, the supernatant was recovered and the oxalic acid extraction was repeated four times. After clearing of acid and concentrating by using a microcentrifuge tube fitted with a filter membrane (Microcon device, MWCO 15000, Amicon Inc.) the elastin content was measured using a Fastin (Biocolor Ltd., Belfast, North Ireland) Elastin Assay kit according to the manufacturer's specifications. The elastin content was expressed as micrograms elastin per milligram protein.

Proteoglycan/Glycosaminoglycan Extraction and Determination

Tissue samples were thawed, rinsed in PBS and minced. Proteoglycans and glycosaminoglycans were extracted according to the method of Carney [Carney, 1987]. Briefly the samples were extracted in 4 M guanidine HCl/0.05; sodium acetate (pH 5.8) containing 6-aminocaproic acid, 10 mM EDTA, 5 mM benzamidine–HCl, 10 mM *N*-ethylmaleimide, 0.4 mM pepstatin, 1 mM PMSF, soybean trypsin inhibitor (1 μg/ml) (Sigma). The samples were nutated at 4°C for 48 h. After dialysis (MWCO 10,000) against PBS, the extracted proteoglycan/glycosaminoglycan content of the samples was determined with a Blyscan (Biocolor Ltd., Belfast, North Ireland) proteoglycan/glycosaminoglycan assay kit according to the manufacturer's instructions. The proteoglycan/glycosaminoglycan content was expressed as micrograms per milligram protein.

Extraction of Tissue Samples for MMP/TIMP Assays

Samples from the native and tissue-engineered pulmonary artery walls were thawed,

rinsed in PBS and minced on ice in extraction buffer containing 2 M NaCl, 10 mM Hepes (2-hydroxyethyl)-piperazine-(ethanesulfonic acid) (HEPES), pH 7.60, and 0.02% NaN_3 (Sigma) as previously described [Moses and Shing, 1994]. After mincing, the samples were nutated at 4°C for 48 h. After centrifugation at 15,000*g* for 15 min the supernatant was filtered through a Nitex membrane.

Substrate Gel Electrophoresis (Zymography)

The presence and activity of gelatinases were determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) copolymerized with gelatin. Gelatin (Type I) was added to standard Laemmli acrylamide polymerization mixture to a final concentration of 1 mg/ml [Herron et al., 1986; Braunhut and Moses, 1994]. Tissue extracts were mixed with substrate gel buffer (10% SDS, 4% sucrose, 0.25 M Tris–HCl, pH 6.8, 0.1% bromophenol blue), loaded into wells of a 4% acrylamide Laemmli stacking gel on a Mini-Protean II apparatus, and run as previously described [Braunhut and Moses, 1994]. After electrophoresis, gels were incubated in 2.5% Triton X-100 with gentle shaking for 30 min at room temperature and one change of detergent solution. Gels were rinsed and incubated overnight at 37°C in substrate buffer (50 mM Tris–HCl buffer, pH 8, 5 mM $CaCl_2$, and 0.02% NaN_3, Sigma). After incubation gels were stained for 15–30 min with 0.5% Coomassie Blue R-250 in acetic acid–isopropyl alcohol–water (1:3:6). Gels were destained in water and photographed. Clear zones against a background of the dark-stained gel indicated proteolytic activity.

For determination of the activity state of MMPs, samples were exposed to 4-aminophenylmercuric acetate (APMA, 1 mM) as previously described [Herron et al., 1986], and electrophoresed as described above. Density of proteolytic bands was measured by scanning densitometry according to manufacturer's instructions using an IS-1,000 Digital Imaging System (Version 2.0; Alpha Innotech, Torrance, CA).

Radiometric Metalloproteinase Assay

MMP-1 and TIMPs activities were determined using a modification [Moses et al., 1990] of the method of Johnson-Wint [1980]. Tissue samples, extracted as previously described, were concentrated 10-fold using a Centricon concentration device (Amicon) according to manufacturer's instructions, and then dialyzed against collagenase assay buffer (50 mM Tris–HCl, pH 7.6, 0.2 M NaCl, 1 mM CaCl, pH 7.4) [Moses and Shing, 1994]. Latent collagenase was trypsin-activitated by incubation with one-tenth volume trypsin (1 mg/ml) for 7 min at 37°C. A one-tenth volume of soybean trypsin inhibitor (5 mg/ml) neutralized the reaction. Samples were added to wells containing ^{14}C-radiolabeled type I collagen and incubated for 2.5 h at 37°C. One unit of collagenase activity represents cleavage of 10% of labeled collagen during 2.5 h of incubation. The supernatants containing soluble radiolabeled collagen were transferred to scintillation vials and counted in a Beckman scintilation counter (Model LS-3801).

MMP inhibitory activity was determined by the addition of 100 μl bovine corneal collagenase to an appropriately diluted sample (100 μl) in wells containing ^{14}C-radiolabeled collagen and incubated as above. One inhibitory unit is defined as the amount of sample required to obtain half-maximal inhibition of collagenase activity in 2.5 h at 37°C.

Western Blot Analysis

Extracted native or tissue-engineered samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 15% acrylamide gel. Proteins were transferred to nitrocellulose with a Transblot apparatus (Bio-Rad). The nitrocellulose was then incubated with anti-TIMP antibodies as previously described [Braunhut and Moses, 1994; Moses and Shing, 1994].

Evaluation of Thickness of Conduit Wall

The thickness of the internal conduit wall was determined using a micrometer device (Mitutoyo Micrometer cd-6 inch cs) and expressed in millimeters.

RESULTS

In order to quantitate the progressive tissue formation of our tissue-engineered vascular constructs, we determined the internal thickness of the conduit wall over the observed time. The thickness increased from initially 0.7 mm to finally 2.1 mm (Fig. 1). Collagen as measured by the 4-hydroxyproline assay was not detect-

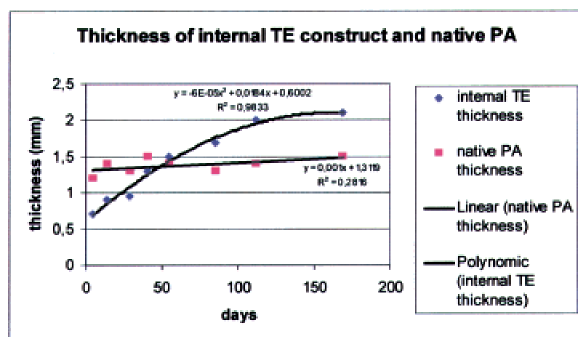
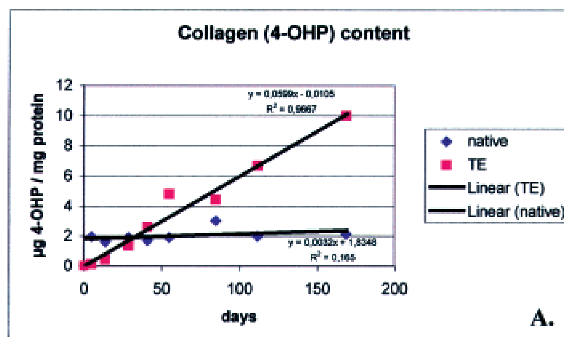


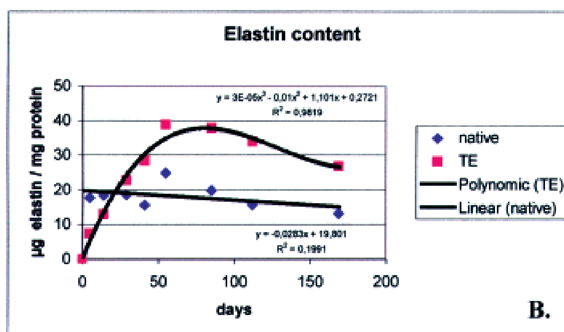
Fig. 1. Thickness of internal conduit wall was determined with a micrometer device and expressed in millimeters. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

able in the control sample (prior to implantation). However, after implantation, the 4-OHP content increased significantly, exceeding the content of the native pulmonary artery with a peak after 169 days (Fig. 2A). Elastin was not detected in the control construct, showed a polynomic increase over the observed time and a peak after 55 days (Fig. 2B).

Proteoglycan/glycosaminoglycan was the only extracellular matrix component to be found in trace amounts prior to implantation. Over the observed time period, this component increased in the TE construct to a maximum at Day 112 (Fig. 2C). As one might expect in a system in which an intense extracellular matrix remodeling is required, we detected a dynamic deposition and remodeling of elastin and proteoglycan/glycosaminoglycan such that at the end of the experiment the amount in the tissue-engineered constructs had either already approached or was approaching that of the native tissue. The exception was the collagen content which motivated us to study the activity of the key player in collagen degradation, the MMP-1 as well as the basement membranes MMP-2 and MMP-9 and their endogenous inhibitors the TIMPs.

A radiometric enzyme assay, using ^{14}C-collagen as the substrate was used to determine activity levels of MMP-1, the matrix metalloproteinase whose activity is specific for Type I collagen. It was important to determine both the pattern of appearance and the activity of this key enzyme in tissue-engineered constructs obtained at various stages of the in vivo studies and equally important to compare these levels to those of the native tissue harvested at the same time. MMP-1 activity over 169 days is





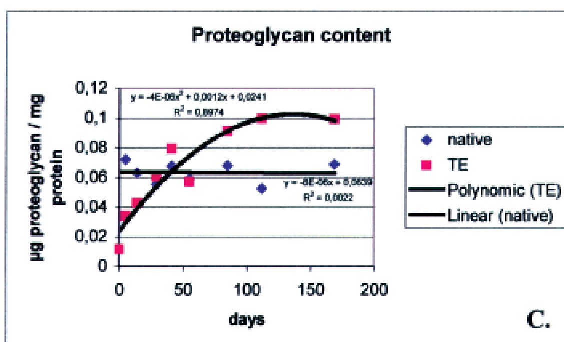


Fig. 2. Assays for quantification of extracellular matrix. All samples were normalized to protein content. (**A**) 4-hydroxyproline (4-OHP) for determination of collagen. (**B**) Elastin. (**C**) Proteoglycans/glycosaminoglycans. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

depicted in Figure 3. By Day 169 there was no significant difference in MMP-1 activity between the native and TE constructs, suggesting that the collagen remodeling in both the native and TE contructs was occurring to a similar degree.

Substrate gel electrophoresis using gelatin as substrate was utilized to determine the levels of activity of MMP-2 (gelatinase A) and MMP-9 (gelatinase B). Equivalent amounts of protein from each TE construct, control and native PA were electrophoresed through the gelatin-impregnated polyacrylamide and devel-

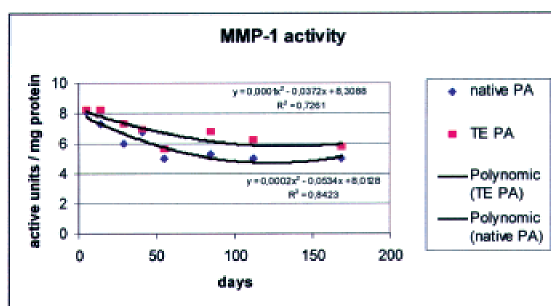


Fig. 3. MMP-1 activity of native (◆) pulmonary artery compared to tissue-engineered artery (■) as determined by radiometric MMP-1 bioassay. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

oped for gelatinase activity. As shown in Figure 4, increased gelatinase activity (MMP-2) was detected during the time period (between days 5 and 85) in which we also observed increases in matrix deposition.

The major species of gelatinase enzymes detected in the samples of interest are the active forms of MMP-2, which migrated at molecular weights of ≈75 and 62 kDa (Fig. 4). As would be expected during a period of intense tissue remodeling, the TE constructs showed increased levels of these proteolytic activities in comparison to their native counterparts up to Day 112 in vivo, by which time the gelatinase activities were comparable in both tissue sets. At Day 169, the TE constructs appeared to exhibit slightly higher levels of enzyme activity.

When levels of MMP inhibitory activities (TIMP) were determined in these identical tissue samples using a radiometric MMP activity assay, we were unable to detect inhibitory activity in either the native or TE samples at any time point with the exception of the Day 169 TE sample. In this sample, we detected an inhibition of 54% (1 Inhibitory Unit/320 μg protein). This is particularly important in that this inhibitory activity was detected in the identical sample which exhibited the increased MMP-2 activity noted above. These data, taken together, suggest that the increased MMP-2 activity in this sample may be balanced by an increase in the endogenous inhibitory activity detected in the same sample.

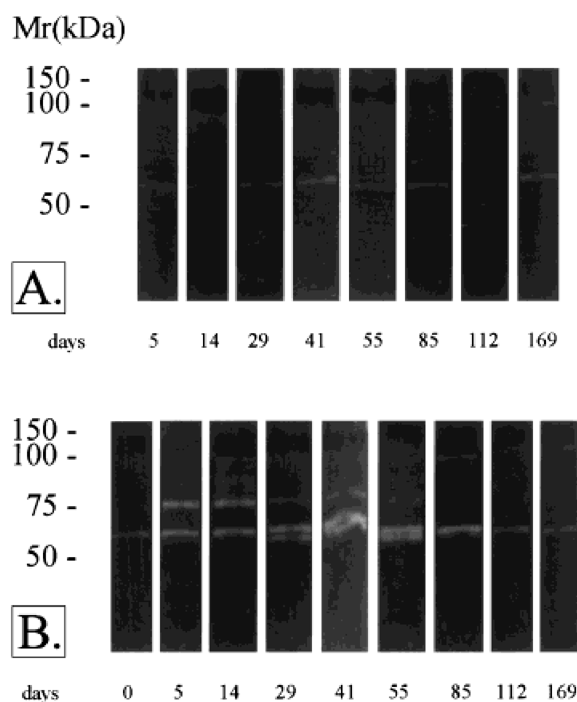


Fig. 4. SDS-substrate gel electrophoresis of tissue samples collected from native (**A**) and tissue engineered (**B**) pulmonary artery prior implantation and at different time points up to 169 days. MMP activities are observed as zones of clearance in a polyacrylamide gel impregnated with gelatin and stained with Coomassie R-250. A band of proteolytic activity was observed at an apparent M_r of 62–66 kDa and corresponds to the active form of MMP-2. An additional proteolytic band at an apparent M_r of 72–76 kDa corresponds to the latent form of MMP-2. MMP-9 is known to show proteolytic activity at an apparent M_r of 92–96 kDa.

DISCUSSION

Pathologic matrix remodeling with excessive extracellular matrix deposition after vascular injury is a well-known clinical entity referred to as intimal hyperplasia. It has been broadly studied in primary arteriosclerosis [Ross, 1990], physiologic adaptation of venous graft material exposed to the systemic circulation [O'Neil et al., 1994], and restenosis after interventional angioplasty [Fuster et al., 1992]. Affected large diameter vessels, such as the aorta, generally show signs of luminal narrowing, flow disturbances and turbulence, but rarely total occlusion. In contrast, small diameter blood vessels, which evidently possess less room for expansion, are much more likely to become occluded during the remodeling process. This fatal outcome is best known and described for the most commonly used small blood vessel substitute, the human saphenous vein. Used as the standard graft for peripheral bypass grafting procedures, they show an average patency rate of 88% after 5 years [Achermann et al., 1998] and

50% after 10 years [Suma, 1999]. Prosthetic devices, such as polyetetrafluoroethylene (PTFE), show an even diminished patency rate of 60% after 5 years [Achermann et al., 1998]. These limitations provide the motivation to use tissue-engineering strategies to build small diameter blood vessels (i.d. < 3 mm) with all properties of native arteries. These properties include a non-thrombogenic internal surface, natural vasoactivity, compliance, and the ability to remodel. For this purpose, tissue-engineering strategies uses cellular components seeded on acellular scaffolds. The seeded cells proliferate and produce their own extracellular matrix. In appropriate cell culture environments, these constructs can resemble native arteries [Shum-Tim, 1997] and are able to response to vasoactive agents and withstand pressure up to 2000 mmHg [Niclason et al., 1999]. Despite early patency, these grafts showed subsequent signs of decreased blood flow and narrowing or occlusion, indicating excessive matrix formation and pathologic matrix remodeling [Shum-Tim, 1997; Niclason et al., 1999]. Unfortunately, this is a common and seriously limiting problem, which must be overcome if clinically useful artificial blood vessels are to be developed.

This study is, to our knowledge, the first to analyze and compare matrix production as well as the enzymes and their endogenous inhibitors responsible for matrix turnover and remodeling in tissue-engineered vascular constructs.

Three important questions regarding matrix remodeling during vascular development were addressed in this study: (1) Is it possible to induce a complex system of physiologic matrix regulation and remodeling in blood vessels grown in vivo after prior in vitro conditioning? (2) If this is possible, how long does it take, to create such a system? (3) Is this turnover mechanism eventually capable of regulating and controlling matrix production after developing morphologic and functional characteristics of a native blood vessel?

In order to address these questions, we chose to use a recently established large diameter (i.d. $= 18$ mm) vascular model in the pulmonary circulation which enabled us to study this complex mechanism for up to 6 months without the danger of occlusion [Stock et al., 2000]. Our results show that the establishment of a complex remodeling system can be achieved in an in vivo environment after proper in vitro conditioning. With respect to the time course of the TE construct remodeling, the data obtained here suggest that it is a long-term process (more than 4 months). Although ECM remodeling as determined by detectable levels of gelatinase activity were observed after 14 days of in vitro culture (prior implantation) at least 169 days were required to accomplish most of the remodeling in vivo.

Quantification of the elastin content during the time course of 24 weeks in vivo demonstrated a kinetic profile that finally resembled that of the native PA. In the case of proteoglycans/glycosaminoglycans, the time course showed an approximation of the profile to the native tissue after 169 days. The collagen determination (4-OHP), however, demonstrated a continuous increase in production and deposition. In order to address this issue, we analyzed activity levels of MMP-1, MMP-2, MMP-9 as well as the activity of TIMPs. There were neither significant differences in MMP-1 activity (radiometric assay) nor any detectable MMP-9 activity by gelatin zymography. We did, however, detect a significant up-regulation of MMP-2 activity between Days 5 and 112. Interestingly, TIMPs were undetectable throughout this experiment with the exception of the final tissue sample. At 169 days, the evaluation of TIMPs showed an inhibitory activity which was matched by increased MMP-2 activity in the same tissue sample.

It remains to be seen whether the continuing collagen deposition that we observed in this study is due to a suboptimal matrix regulation, a pathologic collagen production or both. Inappropriate collagen production is a well-known phenomenon seen in modulated smooth muscle cells. The physiologic, functioning vascular smooth muscle cell (fSMC) contracts, does not proliferate, and produces as much extracellular matrix as necessary to maintain an equilibrium between matrix production and degradation [Chamley et al., 1979]. This fSMC is normally protected by the confluent luminal lining of vascular endothelial cells. However, during arteriosclerotic plaque disruption and angioplasty fSMC are left directly exposed to the shear stress of blood flow. These exposed SMCs, as well as isolated and cultured SMC [Chamley et al., 1979] subsequently modulate to a non-contracting but proliferating phenotype. These proliferative SMC synthesize 25 to

30-fold the amount of collagen compared to contractile fSMC, produce double the amount of synthesized non-collagen proteins [Ang et al., 1990] and fivefold the amount of sulphated glycosaminoglycans [Marilles et al., 1990]. The reversion of this modulation strictly depends on the initial culture conditions. Enzymatic dispersion and high density seeding with an early achievement of confluency enables a return to the contractile state. In contrast enzymatic dispersion with low density seeding or explant culture, as generally used in tissue engineering, result in an irreversible modulation to the synthetic phenotype [Chamley et al., 1977] with all accompanied changes in matrix production. Future studies will address whether the suboptimal collagen deposition that we observed in this study can be attributed, at least in part, to the modulation of SMC.

It is well known that applied shear stress has an important impact on matrix production [Kim et al., 1999]. According to Sterling's Law the wall stress depends on blood pressure, vessels radius and wall thickness (wall stress = pressure) × (vessel radius/wall thickness). Even so the pressure and radius remains constant, the increasing wall thickness results in decreased wall stress. The impact of this decreased wall stress on the cellular response, particularly on the collagen production remains unclear.

In this study, we have determined the remodeling dynamics of tissue-engineered vascular conduits. We believe that the methods and results of this study will serve as a valuable experimental template for the study of tissue-engineered small diameter blood vessel substitutes that must be developed to overcome the current limitation of early occlusion.

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