Tissue Engineering

Vascular Tissue Engineering: Bioreactor Design Considerations for Extended Culture of Primary Human Vascular Smooth Muscle Cells

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The influence of mechanical stimulation on cell populations not only helps maintain the specific cellular phenotype but also plays a significant role during differentiation and maturation of plastic cells. This is particularly true of tissue-engineered vascular tissue, where *in vivo* shear forces at the blood interface help maintain the function of the endothelium. Considerable effort has gone into the design and implementation of functional bioreactors that mimic the chemical and mechanical forces associated with the *in vivo* environment. Using a decellularized *ex vivo* porcine carotid artery as a model scaffold, we describe a number of important design criteria used to develop a vascular perfusion bioreactor and its supporting process-flow. The results of a comparative analysis of primary human vascular smooth muscle cells cultured under traditional"static conditions" and "dynamic loading" are described, where the expression of MMP-2 and 9 and cathepsin-L were assessed. Continued design improvements to perfusion bioreactors may improve cellular interactions, leading to constructs with improved biological function. *ASAIO Journal* 2007; 53:623–630.

A core component of the tissue engineering approach is the bioreactor, a device specifically designed to nurture and stimulate the development of engineered tissues. Perfusion-based culture systems have a number of advantages over traditional static cell culture systems, since complex chemical and mechanical conditions essential to appropriate development can be better controlled.[1] One of the fundamental technical requirements for bioreactors is the system must transfer a homogeneous mix of nutrients, wastes, and gases through the scaffold to promote cell growth and development. To achieve this, a pressure gradient across the scaffold is generated to produce relative high and low pressure circuits. Also important is that the artificially induced shear forces resulting from pressure gradients are controlled to prevent stress-related damage to attached cells. Ideally, the reactor must convey to the developing construct a mechanical environment similar to the original tissue, *i.e.*, pulsed, variable pressure conditions at an appropriate flow rate, to help maintain cellular phenotype. The system must also be designed for simplicity, such that long-term, sterile tissue culture can be conducted with the potential for scaling up for commercial applications.[2–7]

The importance of the mechanical environment in which cell populations reside has a profound effect on cellular behavior.[2,8–11] If the experimental criterion is to produce neotissue with gene expression patterns similar to the original tissue, both complex culture media and mechanical stimuli are required for effective tissue growth. It is important to consider that cells persist in a state of constant flux; molecular attachment to the ECM, cell-cell adhesion, gases, nutrients, and diffusible molecules/ligands modulate cellular phenotype in response to the dynamic and ever changing environment. Mechanical forces contribute directly as the scaffold surrounding the cells expands and contracts moving not only the matrix but the interstitial fluids, as blood shear forces act on the cell surfaces. These shear forces act by stressing a variety of different molecules attached to the cell membrane that transmit via cell signaling cascades or directly through actin filaments or other transmembrane proteins connecting to the cells' interior organelles.[12] Without the application of these dynamic forces, differentiated cells dedifferentiate back to less specialized states and lose functionality. This is typically seen in terminally differentiated primary cells that progressively lose their specific *in vivo* phenotype after prolonged maintenance in traditional static cell culture systems. The use of tissue engineering principles with functional materials and applied mechanical forces promotes the retention of the *in vivo* cellular phenotype.[13–16]

The bioreactor and support infrastructure must transfer to the scaffold, conditions that closely mimic *in vivo* environment to promote phenotype stability, and, ideally, the potential to be scaled-up for clinical applications. The objective of these investigations was the development of a pulsatile perfusion system that emulates human small arterial blood flow, pressure, and pressure waveform, with the ability to modulate frequency and pressure in both lumenal and ablumenal flow circuits. With these diverse criteria, simplicity of design and handling are paramount to minimize the potential for contamination. Using a previously described *ex vivo* scaffold derived from the porcine carotid artery as a model scaffold system,[17] we describe the development of a vascular perfusion bioreactor, its

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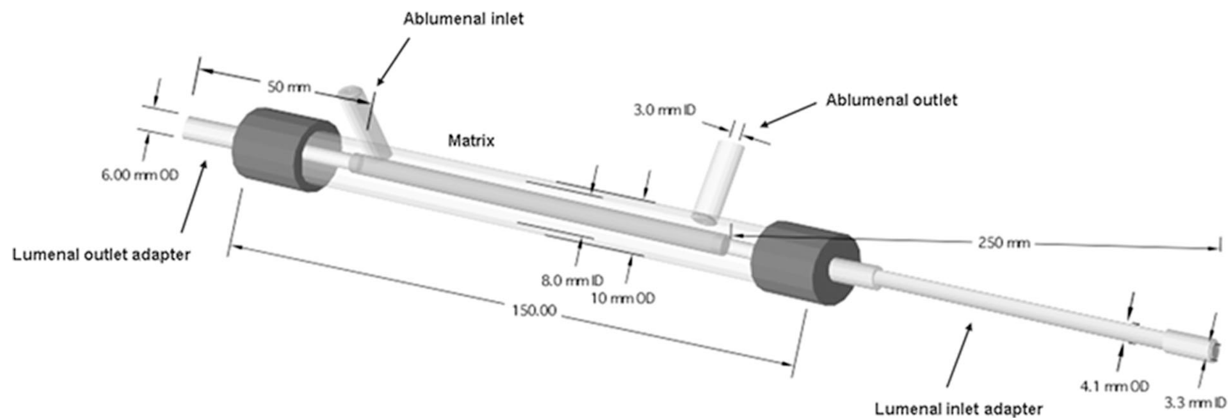

Figure 1. Illustration of the vascular perfusion bioreactor displaying inlet and outlet ports of the reactors main body with a construct mounted between the lumenal inlet and outlet ports.

supporting process-flow, and the resulting effects on cultured vascular smooth muscle cells (VSMC). Established cell seeding techniques were used to adhere "sheets" of primary human VSMC to the scaffold,[18] followed by an evaluation of MMP-2 and 9 and cathepsin-L after 5 weeks of either static or dynamic culture.

Materials and Methods

All chemicals were obtained from BDH (Poole, UK) unless stated otherwise.

Bioreactor Design and Development

The bioreactor was constructed by using glass tubing with Quick-fit 10/2 threaded screw-cap couplings (BDH) to seal the luer-type adaptors that connected to the artery/matrix. Glass ports (3-mm ID) were fused into the main body of the reactor diametrically opposite each other for the ablumenal flow circuit (**Figure 1**). The main body of the reactor was 10 mm (OD) × 8 mm (ID) × 180 mm long, resulting in an ablumenal volume 10.1 cm^3. Inlet and outlet ports were constructed by using 4.1 mm OD × 3.3 mm ID stainless steel with luer-type adaptors (London Surgical, London, UK). Using conditions described in cell seeding and culture, the Reynolds number (N_{Re} = 801) was well within the laminar range. The entry length was calculated by using the "entry port flow conditioning" equation[1] to ensure that fully developed laminar flow entered the bioreactor.[19]

$$L_{ent}/D = 0.370 \exp(-0.148Re) + 0.0550Re + 0.260$$

where D = tube diameter and L_{ent} is the flow conditioning entry port length required to ensure 99% of fully developed flow into the reactor. Under these conditions, an entry length of 146.3 mm was calculated, with the final design length extended to 250 mm to allow for variation in future experimental flow conditions. A dual circuit process flow delivered media to the ablumenal and lumenal surfaces of the scaffold (**Figure 2**). Media was drawn and pulsed by a peristaltic pump (503U Watson Marlow, Falmouth, UK). A compliance chamber was added to reduce system noise/vibration that resulted from the roller action of the peristaltic pump head and to modify the pressure waveform. A one-way check valve with 0.33 psi cracking pressure (Swagelok, Crewe, UK) was added to the lumenal flow circuit to prevent reverse flow and further modify the pressure waveform.

Pressure Waveform Analysis

Real-time pressure and waveform analysis and pressure monitoring were assessed by using 7 bar pressure transducers connected into each flow circuit immediately distal to the bioreactor (**Figure 2**). Each pressure transducer was wired to a PCLD-8115 data acquisition terminal wiring board, connected via a DB3 cable assembly to a PCL-818L computer interface card (Advantech, Milton Keynes, UK); data acquisition was assessed by using VisiDaq software (VisiDaq, Lakewood, NJ). **Figure 3** shows progress made toward replication of the peripheral arterial pressure waveform by modification of the flow circuit to improve flow conditions.

Primary Cell Isolation and Culture

Primary human vascular smooth muscle cells (hVSMC) were isolated as previously described[20] and maintained with Dubelco's Modified Eagle's Medium containing sodium pyruvate, L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco Life Technologies, Paisley, Scotland, UK), and 10% fetal calf serum (Globepharm, Surrey, UK). All cells were maintained at 37°C in a 5% CO_2 environment and used between passages 2 and 4. Cells were phenotypically assessed before seeding for their spindle-shaped, characteristic "hill and valley" formations and for hVSMC-specific antigen α-actin.

Scaffold Preparation

Scaffold processing and analysis have been described previously.[17] Briefly, carotid arteries from 6- to 8-month old Great White pigs were obtained from a local abattoir (Bath Abattoir, Bath, UK). Warm ischemic time was no more than 1 hour from the time of tissue extraction to processing or storage at –5°C. Arteries were 80 to 110 mm in length with lumen diameters tapering from 6.5 to 2.5 mm. Excess connective tissue was removed from the adventitial surface prior to immersion in 75% EtOH with a solvent to tissue mass ratio of 20:1 (vol/wt)

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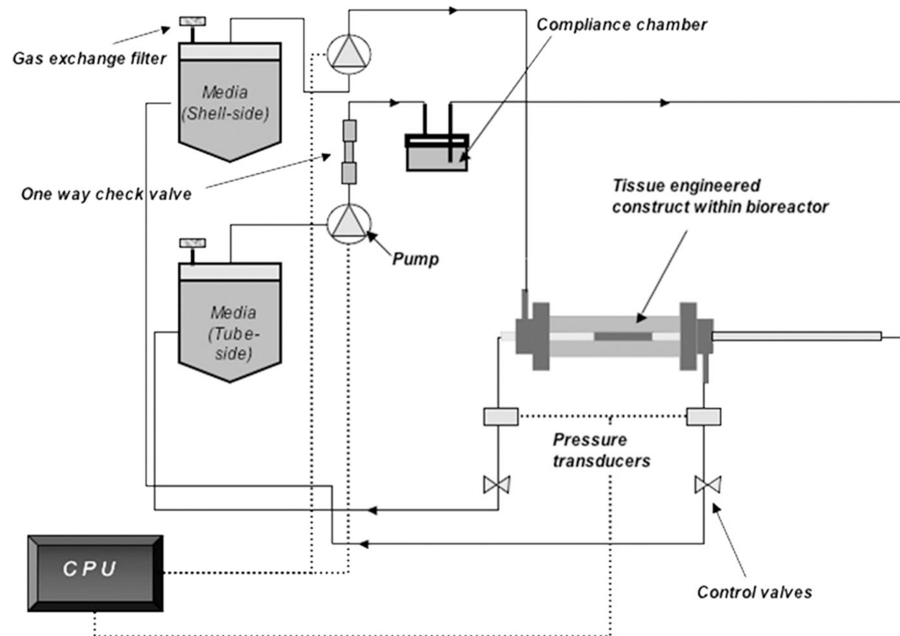

Figure 2. Schematic of the final process flow circuit, illustrating triple reactors in series. The compliance chamber serves to reduce system "noise" to improve the flow regime. The dual circuit design allows full control of media types/composition, pulse frequency, pressure, and flow as independently controllable variables. Pressure is was monitored at the distal end of both the lumenal and ablumenal flow circuits.

in an orbital shaker for 48 hours. The scaffold was then incubated in 1× trypsin-EDTA (Gibco-Life Technologies) prepared in a 1:15 ratio of tissue weight (g) to trypsin volume (mL) for 48 hours. The scaffold was then cross-linked according to the method described by Mechanic.[21] Briefly, the tissue was incubated for 24 hours in a 0.5 M sucrose solution, pH 7.4., then incubated in the cross-linking solution comprised of 0.1% w/v methylene green (Sigma, Dorset, UK) in 4 mol/L NaCl, buffered with 100 mmol/L $NaPO_4$ (pH 7.4) for 24 hours. Tissue was then exposed to broad band light (300 W halogen light source (Lewden, London, UK), suspended 15 cm above the surface of the reaction solution for 22 hours at 0 ± 0.5°C. Air (10 mL/min) was sparged through the stirred reaction solution. Vessels were washed in 1 L volumes of sterile distilled water. Cross-linking and mechanical analysis of the porcine derived material has been previously described.[17,21]

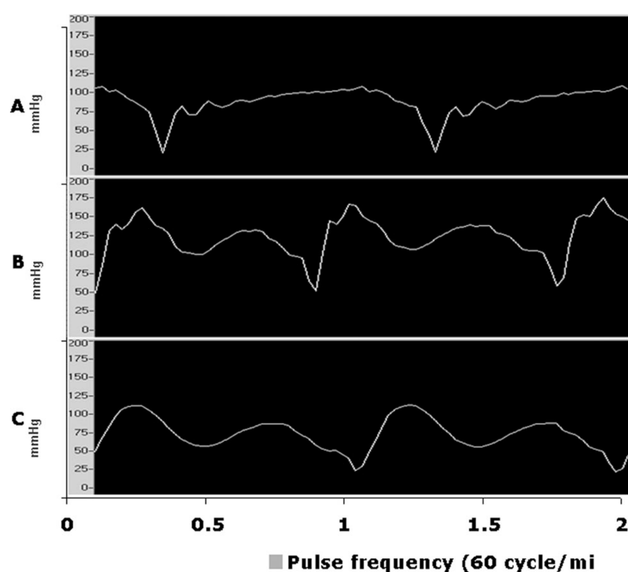

Figure 3. A illustrates the basic pulsatile pressure wave characteristics of the Watson-Marlow 503U peristaltic pump when integrated into the flow system. B, Addition of a compliance chamber alters both the waveform and removes much of the system noise. C, To prevent backflow, a one-way check valve (cracking pressure of 0.33 psi.) was integrated into the flow system immediately after the peristaltic pump.

Scaffold Preconditioning and Seeding

Before cell seeding, the system was sterilized by perfusing a solution of 0.1% (vol/vol) peracetic acid and 4% (vol/vol) ethanol in distilled H_2O, at a rate of 99.25 mL/min at 37°C, 5% CO_2. After 3 hours, the system was flushed with PBS until pH stabilized at 7.4.[22] Matrices were then preconditioned by perfusing 150 mL of SMC media for 48 hours at 33 mL/min, with a pulsation frequency of 0.33 Hz, 37°C, 5% CO_2. All preconditioning media were drained and discarded immediately before cell seeding. Scaffolds were then removed from the bioreactors and seeded.

Static Cultures

hVSMC cultures were bulked up in the normal fashion to produce cell dense layers in T-75 tissue culture flasks. Cells were then incubated in trypsin (1×) until cell sheets began to lift off the plastic substratum, at which point the trypsin was removed and residual trypsin inactivated with the addition of culture media with 15% bovine serum. A cell scraper was then used to remove sheets of cells. Sections of processed matrix approximately 5 × 5 mm were placed in 24-well culture plates (ablumenal surface uppermost) and preconditioned with SMC media for 48 hours at 37°C, 5% CO_2 before placing cell sheets on top of the scaffold sections. These were cultured in minimal media for 2 weeks to allow cell adhesion before applying normal levels of media and culturing for a further 3 weeks, for a total of 5 weeks.

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Table 1. Minimum and Maximum (cyclic) Pressure, Mean Flow Rate, and Pulse Frequency of the Perfusion Cultures Throughout the 5-Week Culture Period

Circuit	Minimum Pressure (mm Hg)	Peak Pressure (mm Hg)	Flow Rate (mL/min)	Pulse Frequency (Hz)
Culture period (weeks 3 to 5)				
Lumenal	0.5	5.0	10.0	0.08
Abluminal	0.0	2.5	0.5	0.004
Culture period (weeks 1 to 2)				
Lumenal	25.0	115.0	165.0	1.33
Abluminal	0.0	2.5	0.5	0.004

Weeks 1 to 2 flow rates were significantly lower to allow cells to adhere without excessive dynamic stress, whereas weeks 3 to 5 were maintained under conditions similar to *in vivo* characteristics.

Perfusion Cultures

Similar conditions were used for seeding onto the 3D surface of a cylindrical scaffold, as described previously,[18] hVSMC were wrapped as cell "sheets" around the scaffold ablumenal surface to bind and generate around the periphery of the scaffold. Once assembled, an initial flow rate of 10 mL/min was delivered through the lumen to allow cell sheets to bind the scaffold for 2 weeks, flow conditions were then adjusted to 165.5 mL/min (1.33 Hz) for the remaining 3 weeks (total 5 weeks). The ablumenal flow circuit was maintained at a flow rate of 0.5 mL/min for the duration of each experiment. Each flow circuit had an excess of 500 mm of tubing to allow fresh tubing to be moved into the roller heads of the pumps to ensure flow and pressure remained stable throughout the 5-week period. Characterization of pressure conditions in each flow system are shown in **Table 1**, with the final pressure waveforms shown in **Figure 3**. Minor variability in the pressure conditions were monitored and corrected by adjusting the distal flow controllers as necessary.

Scanning Electron Microscopy

Low-temperature scanning electron microscopy (LT-SEM) was performed using a JEOL JSM-6310 SEM (Jeol, Tokyo, Japan). Specimens were rinsed (3×) with PBS to remove protein residues from the media, then fixed with 1% (vol/vol) SEM grade glutaraldehyde for 2 hours. After fixation, specimens were mounted and gold-coated. During analysis specimens were maintained at –170°C and viewed using standard procedures at 10 to 15 kV.

Immunohistochemistry

Three sections were dissected from each scaffold, one from each end (10 mm from end) and a third from the mid-section. Prepared samples were washed 2 × 5 minutes in PBS and mounted using DAKO fluorescent mounting medium (DAKO, Cat No. S3023, Ely, UK). Primary antibodies were goat anti-cathepsin L (S-20) polyclonal antibody (Santa Cruz Biotechnology Inc., Cat No. SC-6500, Santa Cruz, CA) used at 1:100 dilution, MMP-2 mouse anti-human-MMP-2 monoclonal antibody (Cat. MAB3308, Chemicon, Chandlers Ford, UK), and MMP-9 mouse anti-human-MMP-9 monoclonal antibody (Cat. MAB3309, Chemicon). Secondary antibodies were mouse anti-goat rhodamine conjugated secondary antibody with an absorption peak at 550 nm and an emission peak at 570 nm (Cat, AP300R, Chemicon). MMP-2 and MMP-9 utilized a horse anti-mouse-FITC conjugate with an absorption peak at 490 nm and an emission peak at 525 nm (Cat. 5024, Chemicon). Visualization was carried with a Carl Zeiss Axioskop2 florescent microscope at the appropriate wavelength using image analysis software from Image Associates UK.

Results

hVSMC Cultures: Static Versus Perfusion-Based Cultures

After 5 weeks of culture, SEM surface analysis showed significant differences in cell structure and morphology between static and perfusion-based culture methods. hVSMC cultured under static culture conditions displayed a less uniform surface morphology (**Figure 4A**), whereas cells cultured for the same period of time (5 weeks) and cultured under perfusion conditions displayed a flatter, more uniform surface structure (**Figure 4B**). **Figure 4C** displays an SEM image of the scaffold before seeding cells. The fluorescent nuclear stain D.A.P.I. confirmed the presence of cells shown in the SEM images **Figures 4D** (static culture) and **4E** (perfusion culture). Scaffold histology displayed similar results for cells cultured under either static or perfusion systems. **Figure 5** shows hVSMC after 5 weeks of static culture having migrated as much as 200 μm into the acellular scaffold via the cut surface, not from the ablumenal surface. Only the static cultures displayed this pattern of migration, as scaffold sections were cut into 5 × 5 mm sections, leaving cut ends available for cells to migrate between the collagen fibers. No cut ends were exposed in perfusion cultures as whole vessels were used (the end sections were discarded as securing clamps compressed the scaffold). Cells cultured under both methods migrated from the scaffold's ablumenal surface only as far as the medial/adventitial interface; no cells were observed to have migrated past this point (**Figure 6**).

Localization of MMP-2, MMP-9, and cathepsin-L expression by hVSMC using immunohistochemical techniques have shown differential expression patterns between static and dynamically stimulated cultures at the end of 5 weeks of culture. Positive controls were obtained by omitting the primary Ab step during the staining protocol (**Figure 7**, I, III, V, and **Figure 8**, I, II, V). Negative controls of the acellular scaffold displayed no Ab localization (data not shown). **Figure 7** shows cells cultured under static conditions with control displaying minimal nonspecific staining in sections where seeded hVSMC are known to reside within the adventitial layer, as shown by hematoxylin and eosin staining (**Figure 6A**). **Figure 7**, II and IV, show

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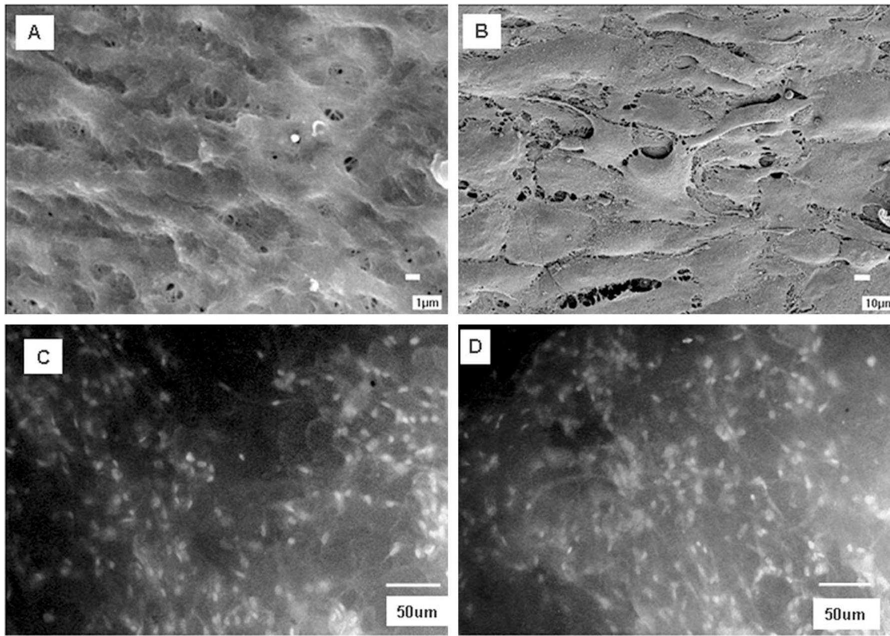

Figure 4. (A) SEM images of hVSMC cultured on the ablumenal surface of the matrix after 5 weeks static culture and (B) after perfusion culture (2 weeks static + 3 weeks perfusion-pulsed flow) show cells in near confluent layers over the surface of the matrix. Distinct differences are noted between the two culture methods. (C) static culture (D) perfusion culture show the presence of DAPI-stained hVSMC nuclei on the scaffold surface.

positive staining for MMP-2 and MMP-9, and **Figure 7**, VI, positive for cathepsin-L expression. **Figure 7**, VI, displays the localization of cathepsin-L in a circular pattern speculated to be the periphery of cell structures (B). The presence of cells corresponds with areas of immunolabeled enzyme (A) within the large cellular aggregates present on the cut surface of the scaffold. Expression of MMP-2, MMP-9, and cathepsin-L by hVSMC after 5 weeks of dynamic cultured conditions is shown in **Figure 8**. Both MMP-9 and cathepsin-L show a strong positive localization of the gene product within the adventitial layers of the scaffold (**Figure 8**, IV and VI), whereas MMP-2 was not localized (**Figure 8**, II). Like static cultures, these data are supported by hematoxylin and eosin staining of hVSMC within the adventitial layer of the scaffold (**Figure 6**, A and B).

Discussion

Cell culture technology over the last 30 to 40 years has been profoundly affected by cell biologists' ability to culture specialized cells in the laboratory. However, even with the use of complex media, terminally differentiated primary human cells progressively lose their specific phenotype after prolonged culture. It is clear that physical stimuli such as scaffold structure, mechanical stimulation, fluid flow, and gas concentrations all contribute to the maintenance specialized cellular phenotypes. Appropriate mechanical stimulation can therefore help maintain the *in vivo* cellular phenotype within an *in vitro* culture system to improve the functionality of engineered tissue constructs.[8,12,23–31]

In these investigations, a porcine-derived acellular scaffold is used as a model system to assess the functionality of bioreactor system and the effects of dynamic culture on human VSMC. In both static and dynamic systems, cells are predominantly localized on the seeded ablumenal surface, with cells migrating as far as the medial/adventitial boundary, as shown in **Figure 6**, A and B. These images typify the migration pattern, with the maximum distance (from the seeded ablumenal sur-

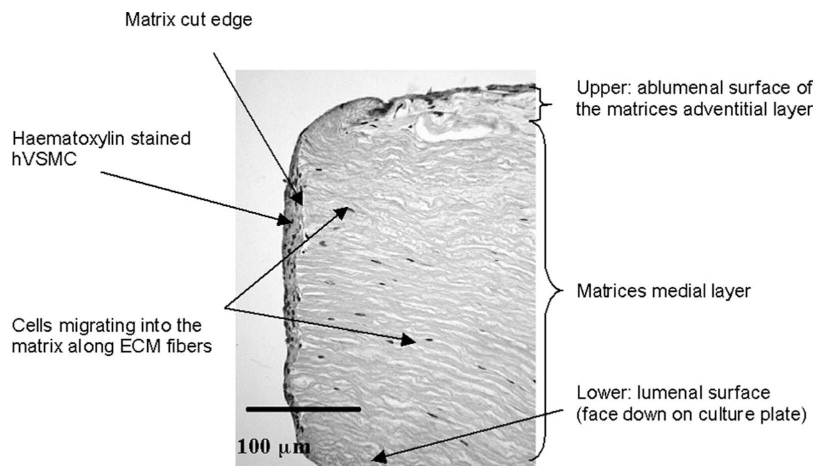

Figure 5. hVSMC seeded onto the ablumenal surface of the porcine-derived arterial matrix and cultured for 5 weeks under static conditions. Sections are stained with hematoxylin, showing hVSMC encasing the matrix and having migrated into the arterial wall (>200 μm) via the cut section of the matrix.

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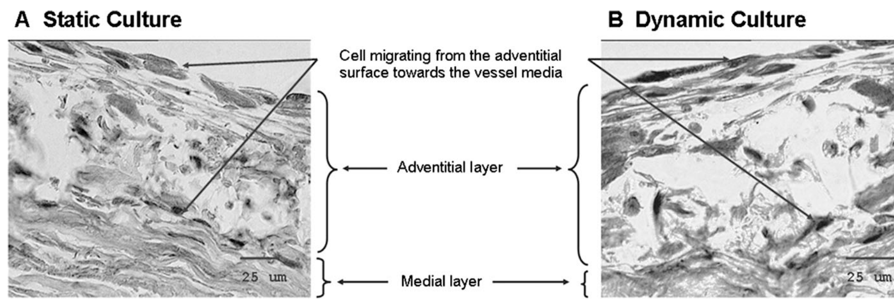

Figure 6. Hematoxylin-stained hVSMC cultured on the ablumenal surface of the scaffold after 5 weeks of culture; (A) static culture, (B) 2 weeks static + 3 weeks perfusion culture. Both static and perfusion-based cultures cells migrated to the adventitial-media boundary; no cells were found to have migrated past this point.

face) ranging between 75 to 125 μm, which is also the approximate distance nutrient diffusion calculated limit to cell function.[32] No cells were observed to cross the medial/adventitial boundary irrespective of distance or culture conditions. Given these results, it was initially unclear if this was a mass transfer limitation where nutrients or other essential supplements were unable to diffuse further into the scaffold, if the cells were not synthesizing the necessary enzymes to degrade the scaffold, or a specific scaffold issue.

To determine whether cells were expressing the necessary enzymes to degrade and remodel the scaffold, the expression of several representative matrix remodeling enzymes (MMP-2 and 9 and cathepsin-L) were assessed. These enzymes are fundamentally important during the coordinated events of matrix degradation and remodeling. Both MMP-2 and 9 have a diverse range of catalytic activity, degrading elastin, a range of collagens and multiple components of the ECM basement membrane.[33] Cathepsin L, a cysteine protease, is generally located inside cellular lysosomes but is also localized outside the cell,[34] also displaying a variety of functions such as degradation of elastin, fibronectin, and several collagen types. As such, the activity of these enzymes is indicative of remodeling activity and is of significance during the development of engineered constructs.[34,35] Although differences appear in the expression profile of MMP-2 between static and perfusion cultures, it is clear that these enzymes are being synthesized, and, as such, they provide the potential for cell migration. Interestingly, cells cultured under static conditions were shown to migrate up to 200 μm into the scaffold from the cut edge of the scaffold, along, and parallel to, the circumferentially orientated collagen fibers. Migration in this direction does not require extensive ECM degradation. These results indicate diffusion was not a limiting factor in the perfusion studies, at least as far as 200 μm into the scaffold. This also

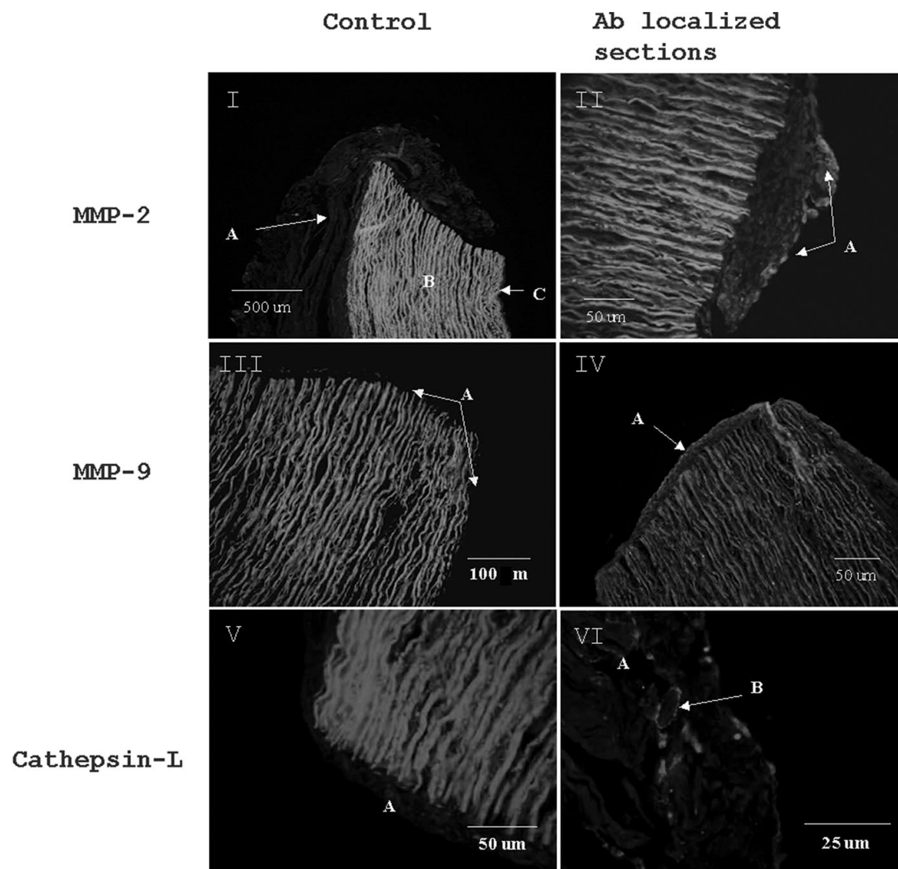

Figure 7. Matrix remodeling static culture: hVSMC after 5 weeks in static culture. I, III, and V controls (no primary Ab) show minimal background or non-specific staining of the hVSMC layer on and within the adventitial layer of the matrix. A indicates areas of seeded cells. II and IV show positive staining for MMP-2 and MMP-9 after 5 weeks of culture within cellular aggregates present on the cut surface of the matrix. VI shows a strong localization of cathepsin-L after 5 weeks in static culture, with some section localizing cathepsin L in a circular pattern speculated to be the periphery of cell structures (B).

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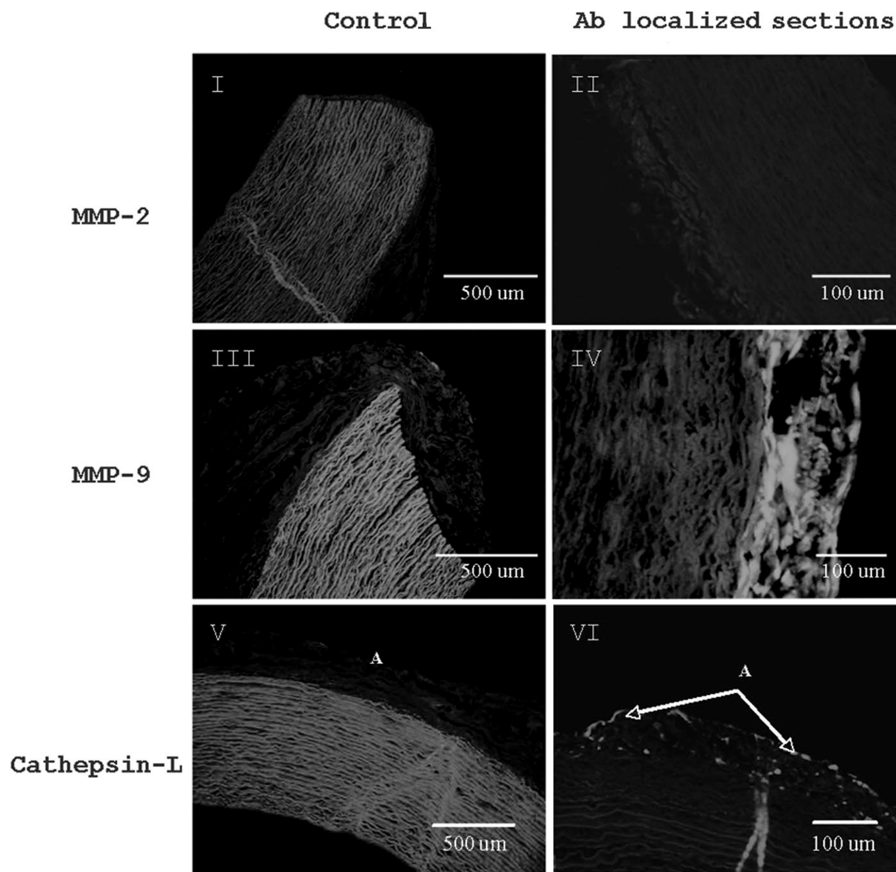

Figure 8. Matrix remodeling perfusion culture: hVSMC after 2 weeks of static culture followed by 3 weeks of perfusion culture. I, III, and V (controls – no primary Ab) show minimal background or nonspecific staining on and within the adventitial layer of the matrix where hVSMC are located (A). II shows negative staining for MMP-2 after 5 weeks of culture, whereas both MMP-2 and cathepsin-L clearly display positive immunolocalization in the outer adventitial layer of the matrix.

provides evidence of an unidentified chemotactic factor that drives cells to migrate deeper into the scaffold. In this light, it could be a cell density issue, where cells at the scaffold interface under several layers of cells may be driven into the scaffold where gas or nutrient levels are more favorable, providing cell density remains low in these areas. From this, we can conclude that cell migration across circumferentially orientated scaffold fibers is inhibited due to the inability of cells to hydrolyze the modified (cross-linked) ECM. Previous investigations have shown this cross-linked scaffold to have threefold increase in resistance to enzymatic digestion compared with non–cross-linked samples.[17] The perceived advantage of many cross-linked materials is that they can limit cell migration to reduce or minimize an immune response. In addition, cross-linked materials have generally been shown to be more stable *in vivo*. The disadvantage with this approach is the inability of cells to fully remodel and populate the material resulting in reduced biological function.

It is clear that mechanical stimulation affects cell function; however, the accuracy with which the *in vitro* system needs to mimic the *in vivo* environment to replicate an appropriate phenotype is not as clear. For example, whether continuous, cyclic, periodic, or random applied stresses result in a similar phenotype is yet to be fully addressed. Clearly, an important advantage of perfusion systems is the uniform convective flow driven by the pressure gradient. A variety of systems have been described in the literature; for a review see Barron *et al.*[7] A significant difference between this system and others described is the ability to control and monitor both ablumenal and lumenal pressures, making possible a more controllable transmural pressure drop. By increasing the pressure in the ablumenal (or shell-side) flow circuit, the transmural pressure differential can be modulated to control transfer through the scaffold. This is likely to be more important for highly porous materials where higher proportional flow permeates the scaffold wall. In addition, if the transmural flow is excessive, cells within the scaffold may be adversely affected by increased shear.

Optimizing the pressure waveform is a more complex parameter to emulate, where subtle changes, for example, in tubing compliance, can have a significant impact on the waveform. In our system, the pressure wave was sequentially modified to emulate a typical diastolic/systolic waveform. Compliance chambers, or pulse dampeners, are commonly used to reduced system noise, dampen and smooth out high/low pressure peaks caused by peristaltic pumps, which (in this case) was caused predominantly by the spring-loaded action of the pump-head rollers. By enclosing a compressible pocket of air within the dampener, the pulsation cycle can be reduced significantly. To prevent backflow, a one-way check valve was inserted into the system after the pump outlet, which also reduced system noise (**Figure 3**).

From a design perspective, vascular bioreactors require a number of specific features due to higher flow and shear conditions to which cells are exposed. Entry ports before the construct, in high-shear systems, would ideally behave (mechanically) similarly to the scaffold material to allow appropriate fluid conditions to interface seamlessly with the de-

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veloping construct. If these conditions are not optimized, such as too-short inlet lengths or abrupt changes in material diameters, cells at the proximal end of the graft will be exposed to disrupted flow, resulting in poor cell adhesion and function. Two critical areas for design consideration are the overall length and thickness of the tube-side inlet ports. Under the conditions described in this paper, the inlet port length was to exceed 140 mm for fully developed laminar flow to enter the bioreactor (**Equation 1**). Our design specified an overall length of 250 mm to account for higher flow rates in future investigations. The second transition point, where the inlet port attaches to the scaffold, the port material's wall thickness must be minimized to reduce diameter change from the port to the scaffold at the transition point; otherwise, significant flow disruption will occur. Ideally, the same "entry length" equation should be applied to the region after this transition point; however, this may not be feasible due to the likelihood of limited cell numbers for these extended graft lengths.

Conclusion

We have developed a modular bioreactor and perfusion system to allow growth and proliferation of vascular constructs over extended culture periods. These enhanced designs, with multiple contiguous functions contained within one system, where tissue processing, cell seeding, culture, and possibly transport, can achieve an effective transition from the research laboratory into the clinic. Unlike more complex designs, an advantage of this compact modular system is the potential to easily convert the bioreactor into a disposable cassette system to further facilitate transfer into the clinical arena. By optimizing both the chemical and mechanical environment in concert with effective materials and culture strategies, improved grafts for reconstructive surgeries may be developed.

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