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Mechanical Properties of Tissue-Engineered Vascular Constructs Produced Using Arterial or Venous Cells

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There is a clinical need for better blood vessel substitutes, as current surgical procedures are limited by the availability of suitable autologous vessels and suboptimal behavior of synthetic grafts in small caliber arterial graft (<5 mm) applications. The aim of the present study was to compare the mechanical properties of arterial and venous tissue-engineered vascular constructs produced by the self-assembly approach using cells extracted from either the artery or vein harvested from the same human umbilical cord. The production of a vascular construct comprised of a media and an adventitia (TEVMA) was achieved by rolling a continuous tissue sheet containing both smooth muscle cells and adventitial fibroblasts grown contiguously in the same tissue culture plate. Histology and immunofluorescence staining were used to evaluate the structure and composition of the extracellular matrix of the vascular constructs. The mechanical strength was assessed by uniaxial tensile testing, whereas viscoelastic behavior was evaluated by stepwise stress-relaxation and by cyclic loading hysteresis analysis. Tensile testing showed that the use of arterial cells resulted in stronger and stiffer constructs when compared with those produced using venous cells. Moreover, cyclic loading demonstrated that constructs produced using arterial cells were able to bear higher loads for the same amount of strain when compared with venous constructs. These results indicate that cells isolated from umbilical cord can be used to produce vascular constructs. Arterial constructs possessed superior mechanical properties when compared with venous constructs produced using cells isolated from the same human donor. This study highlights the fact that smooth muscle cells and fibroblasts originating from different cell sources can potentially lead to distinct tissue properties when used in tissue engineering applications.

Introduction

CARDIOVASCULAR DISEASES are the leading cause of mortality in North America.[1] The gold standard for small caliber blood vessel replacement such as the coronary artery is currently the transplantation of a native autologous graft, such as the saphenous vein or the internal mammary artery.[2] However, the limited availability of healthy and suitable autologous vessels, especially in the case of repeated bypass procedures, is a drawback for further success in this field. Other synthetic materials, such as Dacron and expanded polytetrafluoroethylene, present high risk of thrombosis in the replacement of small caliber blood vessels. In an effort to overcome these limitations, vascular tissue engineering has recently shown great potential in clinical studies aiming at small diameter autologous vascular replacements.[3,4]

The design of a functional tissue-engineered blood vessel (TEBV) has been a challenge for a number of years.[5,6] Since the pioneering work of Weinberg and Bell,[7] several methods were developed to produce tissue-engineered vascular constructs, most of them involving cells incorporated within a variety of biomaterial scaffolds or extracellular matrices (ECM).[8–17] Most of these methods have been reviewed elsewhere.[18] Using these approaches, several cell sources such as endothelial cells (ECs), vascular smooth muscle cells (SMCs), fibroblasts, myofibroblasts, and, more recently, muscle-derived stem cells and pericytes have been used, some of them leading to interesting *in vivo* results.[19–21] To produce a functional TEBV, the use of autologous cells is of great interest, as the deleterious effect of rejection and immunosuppressive medication is avoided.[22,23]

The self-assembly approach is based on the exclusive use of cells combined with their ability to produce an abundant

ECM when cultured in the presence of ascorbic acid. Highly resistant human blood vessels comprising an adventitia, a contractile media, and an intima can be produced using this cell-based tissue engineering method.[24] Functional tissue-engineered vascular constructs can be produced from dermal fibroblasts, saphenous vein fibroblasts, and vascular SMCs.[24–28] These cells are potential sources for the production of autologous TEBV for clinical applications. Based on the literature, native arteries represent a better choice for arterial bypass than native veins.[2] However, the autologous saphenous vein remains a very useful substitute for most surgeons performing these procedures because of limited arterial graft availability. To address problems related to vein graft such as intimal hyperplasia and accelerated atherosclerosis that can result from compliance mismatch at the anastomoses, various biodegradable polymer wraps were proposed as external mechanical supports.[29,30] Thus, it becomes interesting to compare the mechanical properties of tissue-engineered vascular constructs produced using arterial or venous cells to investigate the cell type dependence of the self-assembly approach. To avoid inherent interindividual differences, the experimental design included the isolation of all cell types from an artery and a vein from the same subject.

The aim of the present study was to assess the mechanical properties of engineered tissues to determine whether they are dependent on the source of cells, venous or arterial, used for tissue fabrication. We took advantage of the self-assembly approach to produce autologous tissue-engineered vascular constructs from SMCs and fibroblasts extracted from the artery and the vein of the same umbilical cord, harvested from three distinct donors. The mechanical and viscoelastic properties of these venous or arterial vascular constructs were evaluated and compared by means of uniaxial ring testing, stress-relaxation testing, and cyclic loading hysteresis analysis. Although vascular constructs can be produced from both, venous or arterial cells, their biomechanical and histological properties presented cell-source and cell-type dependent differences. These results suggest that using cells originating from arteries enhances the mechanical performances of self-assembled tissue-engineered vascular constructs.

Materials and Methods

This study was approved by the Centre Hospitalier Affilié Universitaire de Québec (CHA) institutional review committee for the protection of human subjects. Tissues were obtained after informed consent had been given.

Cell isolation and culture

Arterial and venous ECs, SMCs, and fibroblasts were isolated from three distinct human umbilical cords as previously described with some modifications.[31] Briefly, a section of umbilical cord was obtained from a healthy newborn, transported at 4°C in a solution of Dulbecco-Vogt modified Eagle medium with Ham's F12 (DMEM-Ham; ratio 3:1; Invitrogen) supplemented with 10% fetal bovine serum (FBS; Hyclone) and antibiotics (penicillin [100 U/mL; Sigma], gentamycin [25 μg/mL; Schering]) and processed less than 6 h after biopsy sample harvesting. The umbilical cord was washed in phosphate-buffered saline (PBS), umbilical artery

and umbilical vein were dissected out of the cord using a scalpel and scissors without damaging the vascular tissue and were treated separately using the following protocol.

Vascular conduits were cut into approximately 10 centimeter-long sections and carefully rinsed by flowing PBS into their lumen using a sterile syringe (Terumo Medical Corp.). To extract ECs, the vessels were connected at both ends using a two-way stop-cock valve (Cole-Parmer), fixed with a prolene monofilament (Ethicon Endo-Surgery), and filled with a thermolysin solution (250 μg/mL; Sigma) using a syringe. Both valves of the thermolysin-filled vessels were shut, and then, the vessels were put in PBS and incubated at 37°C for 15 min. The thermolysin solution containing ECs was removed by opening the valve and using the syringe piston to evacuate the fluid contained in the vessel.

To extract SMCs and adventitial fibroblasts, the vessel was opened longitudinally, pinned to a dissection board with the lumen facing upward, and gently wiped with a sterile gauze soaked in PBS to eliminate any remaining ECs. Fragments of the thin underlying media layer were then carefully collected with sterile tweezers and fine forceps, cut into smaller pieces, and placed in a gelatin-coated Petri dish (BD Biosciences) to allow the outgrowth and attachment of SMCs. The remaining vessel was turned upside down on the dissection board (lumen facing downward), and the procedure used on the media was repeated on the external component of the vessel to extract the fibroblasts from the perivascular connective tissue. Explants were cultured in DMEM-Ham supplemented with 30% FBS, 20 μg/mL EC growth supplement (ECGS; Invitrogen), and antibiotics until SMCs and fibroblasts migrated out of the biopsy samples.

Tissue samples from each cell isolation phase were processed for histology. Results confirmed that explants were harvested from the intended layer of the blood vessel: endothelium for the ECs, media for the SMCs, and adventitia for the fibroblasts (Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/tea). Two weeks later, SMCs and fibroblasts were trypsinized (0.05% trypsin [Intergen] and 0.01% EDTA [JT.Baker]), plated at 1×10^4 cells/cm^2 density in noncoated tissue culture flasks (BD Biosciences). All cell types displayed a constant proliferation rate and phenotype during the subculture, and immunofluorescent staining showed that both arterial and venous SMCs expressed calponin and desmin, whereas fibroblasts did not express these markers (Supplementary Fig. S2, S3). All cell types were used at passage 5 and were maintained at 37°C in a humidified incubator containing 8% carbon dioxide. Culture medium was changed thrice per week.

Tissue-engineered vascular constructs

Tissue-engineered vascular constructs were produced using an adaptation of the tissue engineering method previously described, namely the self-assembly approach.[26] Arterial or venous SMCs (1×10^4 cells/cm^2) and adventitial fibroblasts (3×10^4 cells/cm^2) were seeded, in two distinct compartments of a gelatin-coated 245 mm × 245 mm tissue culture plate (Corning) separated in half by a custom-designed spacer and cultured in DMEM-Ham supplemented with 30% FBS, 5 μg/mL ECGS, and antibiotics until cells adhered to the underlying gelatin coating. Sodium L-ascorbate (50 μg/mL; Sigma) was added to the culture medium of

every cell type to stimulate ECM synthesis. Twenty-four hours after cell seeding, the spacer was removed to allow the two cell types to proliferate and form a contiguous sheet of tissue containing both SMCs and fibroblasts. Cells were cultured for 14 days until their neosynthesized ECM proteins assembled to form an adherent living tissue sheet. This tissue sheet was then separated into either six distinct 80 mm ×120 mm sheets containing either SMCs or fibroblasts (three of each) or three distinct 80 mm ×240 mm sheets containing both SMCs and fibroblasts. Each individual tissue sheet was gently detached from the culture flask using fine forceps, rolled onto a 4.5 mm diameter polystyrene tubular support, and maintained in culture in DMEM-Ham supplemented with 10% bovine Fetal Clone II serum (HyClone), antibiotics, and 50 μg/mL of ascorbic acid. Arterial and venous tissue-engineered vascular media (TEVM) were obtained by rolling a tissue sheet produced by either arterial (aTEVM) or venous SMCs (vTEVM), whereas tissue-engineered vascular adventitia (TEVA) were obtained by rolling either arterial (aTEVA) or venous (vTEVA) fibroblastic tissue sheets on a tubular support. Tissue-engineered vascular constructs comprised of both a media and an adventitia (TEVMA) and will be referred to as aTEVMA or a vTEVMA, depending on whether the TEVMA was produced using arterial or venous SMCs and fibroblasts.

All vascular constructs were maintained for a 14-day-culture period on the tubular support at 37°C in a humidified incubator containing 8% carbon dioxide. The culture medium was changed thrice per week.

Histology

After a 14-day-culture period on the tubular support, biopsies of each type of vascular constructs were fixed overnight in Histochoice (Amresco) and embedded in paraffin. Five-micrometer thick sections were stained with Masson's trichrome and observed on a Nikon Eclipse TS100 microscope.

Immunofluorescence

Indirect immunofluorescence detection of type I collagen and elastin was performed on frozen sections after fixation in methanol for 10 min at −20°C using either a mouse anticollagen I (Calbiochem) with an Alexa Fluor 594-labeled-donkey anti-mouse IgG (Sigma) or a rabbit anti-human elastin (A. Grimaud, Institut Pasteur, Lyon, France) with an Alexa Fluor 594-labeled-chicken anti-rabbit IgG. Primary antibody was omitted for controls. Immunofluorescence was visualized using a Nikon Eclipse E800 epifluorescence microscope.

Uniaxial tensile testing

Tissue-engineered vascular constructs were subjected to tensile ring testing[15] on an Instron Electropuls mechanical tester (Instron Corporation, Norwood, MA). Constructs were cut into 5 mm ring samples and mounted between two hooks adapted to the mechanical tester. The hook-to-hook distance was determined as the gauge length of the samples. Tissue-engineered samples were preconditioned with three cyclic loading sequences estimated at 10% of failure strain before testing (data not shown). The rings were loaded to failure

with a displacement rate of 0.2 mm/s. Ultimate tensile strength (UTS) and failure strain were defined by the peak stress and maximum deformation withstood by the samples before failure. Linear modulus was defined as the slope of the linear portion of the stress-strain curve comprised between 25% and 80% of the UTS of the sample.[32] Note that engineering stresses were calculated by dividing the recorded loads by the cross-sectional area of the sample using initial construct dimensions and that engineering strain was used to measure the deformation of the vascular constructs. Stress-strain curves were plotted and analyzed using a Matlab® script (The Mathworks) to facilitate the calculation of the tensile testing parameters.[26]

Estimated burst pressure

The estimated burst pressure was calculated from UTS measurements by rearranging the law of Laplace for a pressurized thin-walled hollow cylinder:

$$BP_{(Estimated)} = 2\frac{UTS \cdot t}{ID}$$

where t is the thickness and ID is the unpressurized internal diameter of the vascular constructs.[21,33] Geometry of the construct was based on measurements of tissue thickness on histological cross-sections of the sample and ID was estimated at 4.5 mm, corresponding to the diameter of the tubular support used for vascular constructs assembly.

Stepwise stress-relaxation testing

Stress-relaxation testing of the different vascular constructs was performed as described.[26,32] Briefly, constructs were cut into 5 mm ring samples and mounted between two hooks adapted to the mechanical tester previously stated. Since these tests required an extended period of time, an environmental chamber was designed to allow for the tissue sample to remain in culture media at 37°C during the whole process. Three instantaneous incremental displacement steps of 10% strain were applied between hold periods of 15 min to allow the tissue to reach equilibrium. These timepoints were chosen on the basis of preliminary results demonstrating that peak and equilibrium recorded values measured for each incremental step using these specific parameters followed a linear evolution as a function of time, allowing for a direct measurement of the initial and equilibrium moduli on the stress-relaxation graph.[26] Initial modulus was evaluated by calculating the slope of the best fit of stress as a function of strain following each incremental step, whereas equilibrium modulus was calculated from the slope of the best fit of stress as a function of strain following each relaxation period (Fig. 3A). Stress-relaxation data were plotted and analyzed using a Matlab script, allowing the detection of peak and equilibrium values as well as calculation of the peak and equilibrium moduli.

Hysteresis

Repeated loading-unloading cycles were performed on the vascular constructs to obtain hysteresis stress-strain curves and to assess the elastic behavior of the engineered tissues. Constructs were cut into 5 mm ring samples, were mounted onto the mechanical testing setup previously described, and

were subjected to 20 min of continuous loading-unloading cycles at a frequency of 1 Hz for a total of 1200 cycles. To remain in the toe-region of the stress-strain curve of the tissue, the amplitude of the applied deformation corresponded to 10% strain based on initial construct geometry. These experimental parameters were determined on the basis of an average duty cycle experienced by a blood vessel in normal *in vivo* conditions. Hysteresis curves were analyzed using an in-house developed Matlab script, allowing for the plot of the 1st, 500th, and 1200th loading cycle to monitor the load-bearing capacity of each vascular construct and to denote differences in the elastic behavior between the different tissue conditions.

Statistical analysis

The experiment was performed on vascular constructs produced using arterial and venous cells extracted from three distinct umbilical cords. Three TEVM, three TEVA, and three TEVMA were produced with arterial (nine arterial constructs) and venous (nine venous constructs) cells, and this pertained to cells extracted from three different umbilical cords (3 cords × 18 constructs per cord = 54 vessels total in the experiment). Results are expressed as mean ± standard deviation. In the case of uniaxial ring testing, three ring sections from a same construct were tested for every construct (3 tensile tests per construct × 3 constructs per condition = 9 tensile tests per condition). In the case of ring testing performed on native tissue, a minimum of three samples from both the umbilical artery and the umbilical vein were tested for all three umbilical cords (nine tensile tests total for umbilical artery, *idem* for umbilical vein). In the case of stress-relaxation testing, a single section of each construct was analyzed for TEVM, TEVA, and TEVMA produced with arterial and venous cells (three stress-relaxation tests per condition). The same experimental design was used in the case of repeated loading-unloading cycles where a single section of each construct was tested. Normality was established using the Anderson-Darling test with a standard $p < 0.05$. Comparisons of specific parameters between the arterial and venous constructs within a same condition were performed using a Student-t test. Comparison of the parameters between the different types of constructs produced using a same source was performed using an analysis-of-variance general linear model and a *post hoc* Tukey test. Data were analyzed using Minitab (Minitab). Statistical significance was established using a standard $p < 0.05$.

Results

Histology and immunofluorescence

Both arterial and venous cells were efficient for the reconstruction of self-assembled vascular constructs (TEVM, TEVA, and TEVMA). To evaluate the impact of cell source on the structure and composition of the ECM of the vascular constructs, Masson's trichrome and immunofluorescence staining were performed (Fig. 1). Histology of arterial TEVM (Fig. 1A) presented a denser and more compact ECM when compared with its venous counterpart (Fig. 1J). Immunofluorescence imaging showed that type I collagen is expressed in each vascular construct. In contrast, elastin labeling was expressed in the aTEVM and in the media
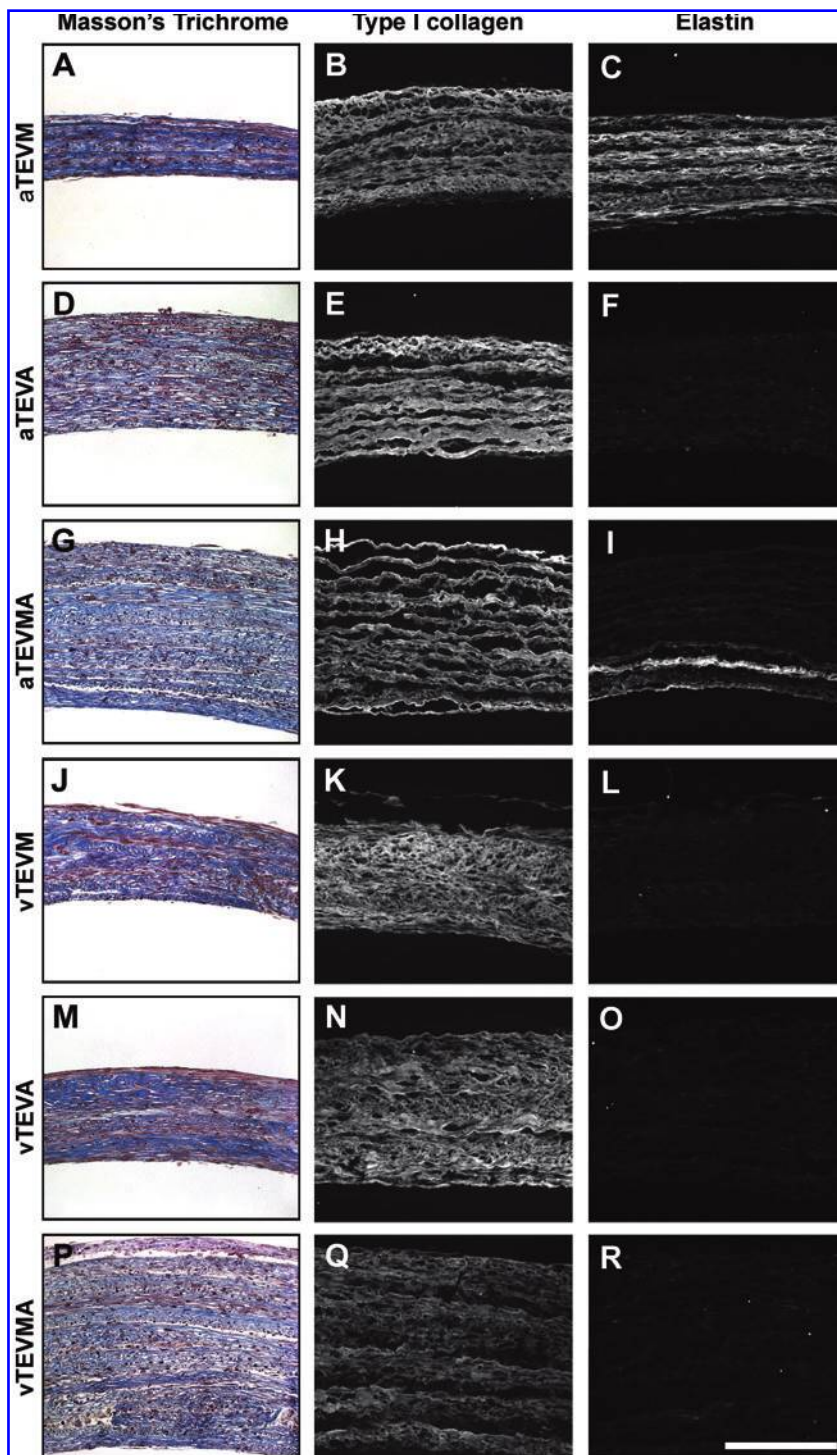
portion of the aTEVMA, suggesting that more elastin is produced by arterial SMCs in these vascular constructs when compared with the other vascular cell types tested in these conditions (Fig. 1C, I).

Mechanical strength

Uniaxial tensile tests were performed to evaluate mechanical strength of the constructs. A characteristic stress-strain profile comprised a toe region, followed by a linear stress-strain relationship, and a rupturing point typical of viscoelastic biological tissues was observed for every sample.[26] A trend in UTS measurements showed that arterial vascular constructs display superior mechanical strength when compared with similar constructs produced with venous cells. A significant difference was observed in the case of TEVM and TEVMA (Fig. 2A). aTEVM and vTEVMA displayed, respectively, significantly higher and lower UTS than the other tissue-engineered vascular constructs. These results appear to be different than values of UTS obtained for native umbilical artery and vein, although these results can be attributable to the difference in thickness observed between these two tissues (not shown). Similar results were seen in the evaluation of the linear modulus, where aTEVA and aTEVMA constructs showed superior values than their venous counterparts (Fig. 2B). The linear modulus measured for native umbilical artery was significantly lower than that of umbilical vein, which was similar to the modulus measured for aTEVMA. All the vascular constructs were able to sustain up to 30% strain without failure. This parameter was not affected by the cell source (arterial or venous) and differed drastically with values obtained for native umbilical blood vessels, as the failure strain obtained for the vein was approximately 150%, whereas the artery was able to deform over 500% without rupturing (Fig. 2C). Based on the estimated burst pressure, no difference was observed in the burst strength of TEVM and TEVA. However, the burst pressures estimated for aTEVMA was significantly higher than that of vTEVMA. Although the estimated value obtained for the aTEVMA is approximately five times lower than that of native artery, the trend observed between the aTEVMA and vTEVMA constructs were in accordance with results obtained for native artery and vein. These observations showed that under the condition tested, the production of vascular constructs using arterial cells results in increased mechanical strength when compared with constructs produced with venous cells.
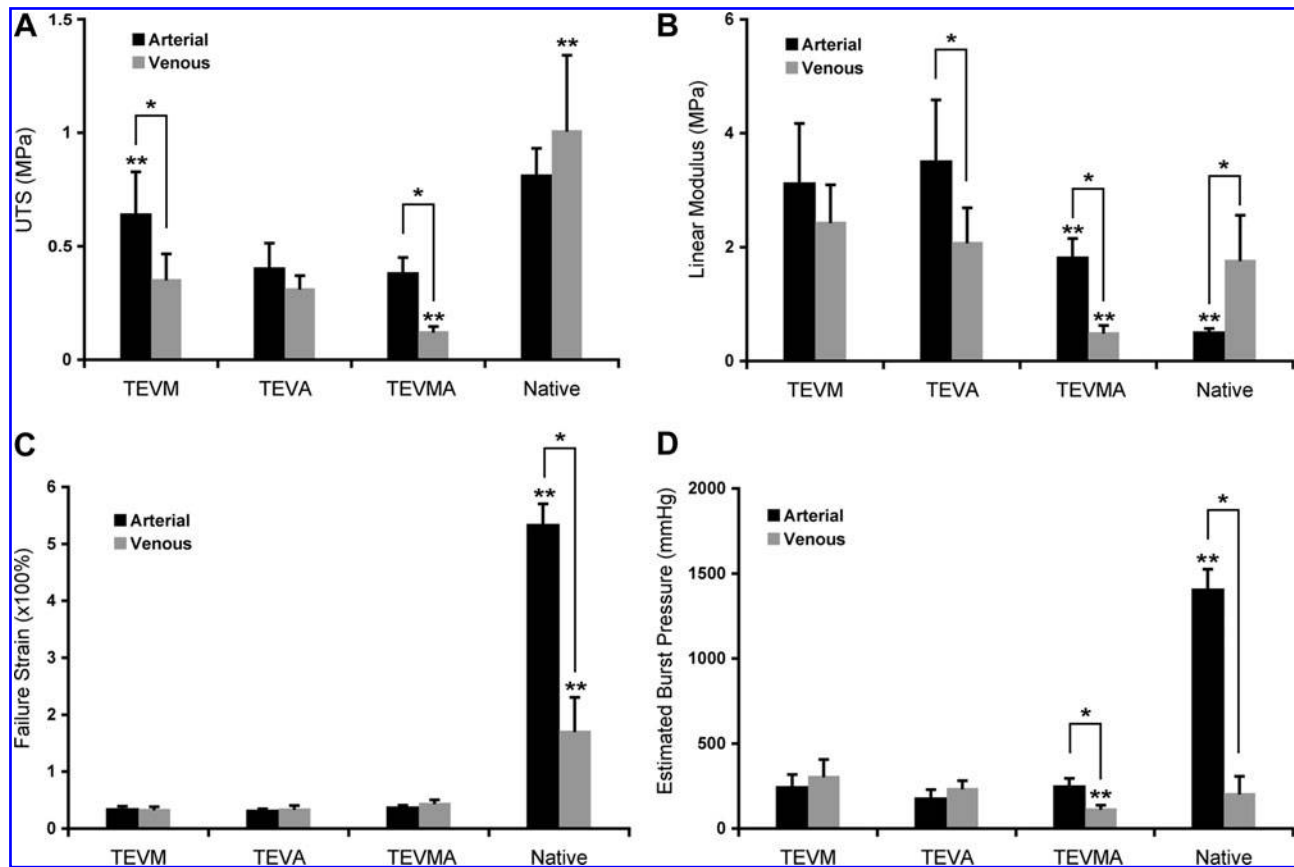
Viscoelastic properties

Stress-relaxation profiles obtained for the vascular constructs were characterized by a peak value, followed by an exponential decay of the load measured in the tissue over time (Fig. 3A). Initial and equilibrium moduli were evaluated to compare the stress-relaxation behavior of the different vascular constructs (Fig. 3B, C). The initial modulus was superior for TEVMA constructs, aTEVMA displaying a significantly higher initial modulus than vTEVMA (Fig. 3B). Similarly, the equilibrium modulus measured for the aTEVMA was significantly superior to the vTEVMA. These results indicate that under these conditions, TEVMA produced using arterial cells have the capacity to bear and withstand higher loads for the same amount of deformation than those

FIG. 1. Histological cross-sections stained with Masson's trichrome **(A, D, G, J, M, P)** and immunostaining of type I collagen **(B, E, H, K, N, Q)** and elastin **(C, F, I, L, O, R)** of the tissue-engineered vascular constructs. Note that collagen I expression is found in each type of vascular constructs, whereas elastin expression is increased within aTEVM and aTEVMA comprising arterial smooth muscle cells **(C, I)**. Scale bar: 250 μm. aTEVM, arterial tissue-engineered vascular media; aTEVMA, arterial TEVM constructs comprised of both a media and an adventitia. Color images available online at www.liebertonline.com/tea

produced with venous cells. The modulus ratio was evaluated to determine whether constructs are displaying viscous or elastic attributes (Fig. 3D). All the modulus ratios evaluated were comprised between 1.3 and 1.8, therefore indicating that every type of vascular constructs displayed elastic attributes. The modulus subtraction represented the difference between the initial and equilibrium modulus and was calculated to evaluate the differences between the viscoelastic behaviors of the different constructs (Fig. 3E). Modulus subtraction results showed that all vascular constructs

were able to sustain loading for an extended period of time. The combination of modulus ratio and subtraction results indicated that all vascular constructs are displaying an elastic behavior. This observation is in accordance with hysteresis curves obtained after cyclic loading of the vascular constructs (Fig. 4). Indeed, each vascular construct was able to endure 1200 loading-unloading cycles without experiencing continuous creep, and they all reached a state of equilibrium between the 500th and 1200th loading cycle. Moreover, results showed that TEVM, TEVA, and TEVMA produced

FIG. 2. Mechanical properties of the tissue-engineered vascular constructs compared with native tissue. Bar graphs representing ultimate tensile strength (UTS) **(A)**, linear modulus **(B)**, failure strain **(C)**, and estimated burst pressure **(D)** evaluated for vascular constructs produced using arterial cells (black) or venous cells (gray) and for native umbilical cord artery (black) and vein (gray). Note that both the UTS and the linear modulus increased for vascular constructs produced using arterial cells compared with engineered tissues produced using venous cells. Failure strain was not affected by the cell source used for the production of vascular constructs, but differed significantly from failure strain of both arterial and venous native tissues. Estimated burst pressure displayed a significant difference between arterial and venous cell sources in the TEVMA condition and in the native tissue condition. Results are expressed as mean ± standard deviation. *Statistical significance between arterial and venous parameters within the same condition for engineered and native tissues ($p < 0.05$). **Statistical significance for comparison to other arterial or venous conditions ($p < 0.05$).
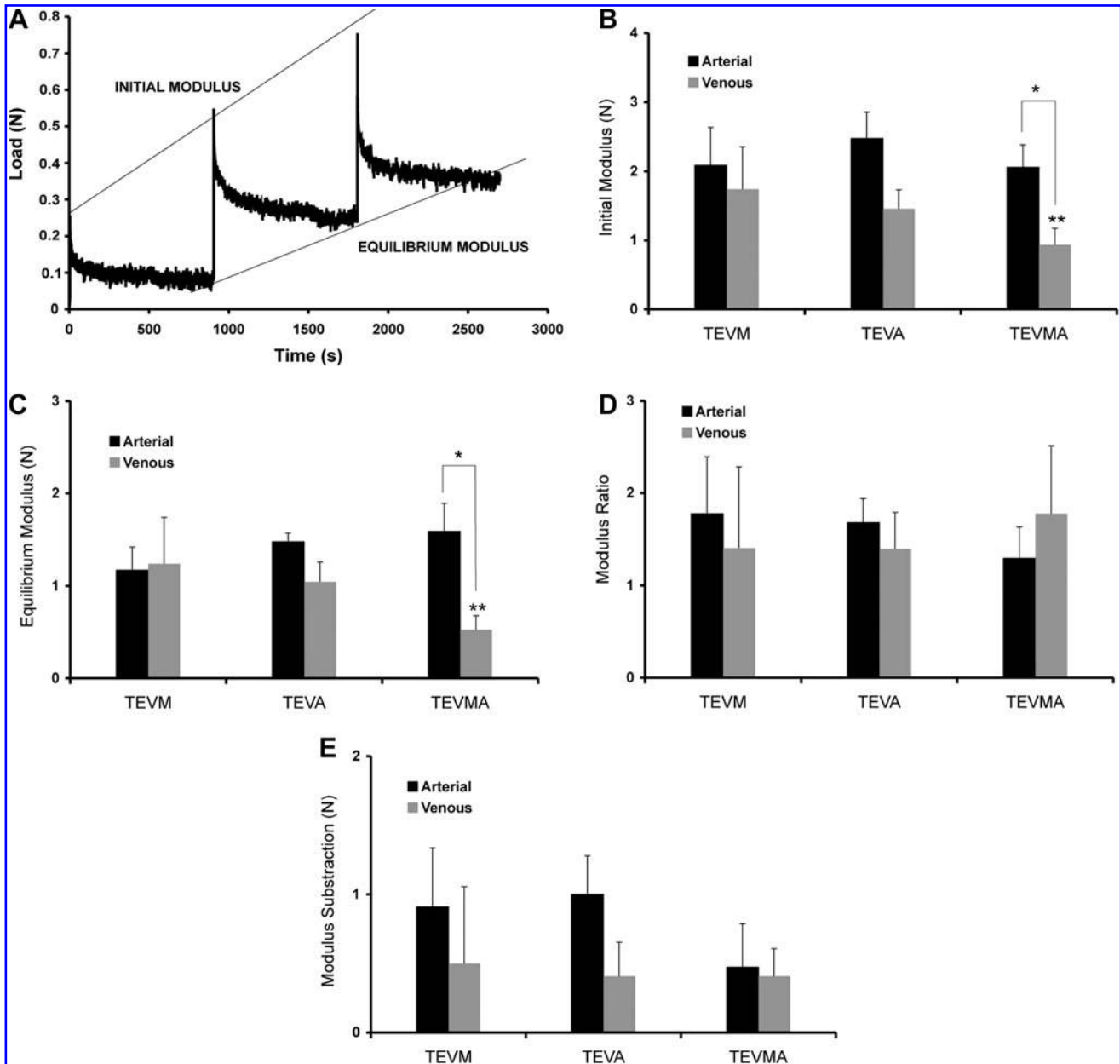
using arterial cells (Fig. 4A, C, E) have increased load-bearing capacity when submitted to 10% cyclic strain, as they reach equilibrium at higher loads than their venous counterparts (Fig. 4B, D, E). In summary, viscoelastic testing indicated that both arterial and venous constructs display elastic attributes, an important parameter to ensure adequate mechanical function of a TEBV.

Discussion

This work highlights the ability of the self-assembly technique to produce multiple arterial and venous tissue-engineered autologous vascular constructs from a single tissue source. Both arterial and venous cell types were able to produce and assemble ECM components to form a functional vascular construct. Interestingly, elastin expression was increased in tissue comprising arterial SMCs, and this observation was reflected in the cyclic mechanical behavior of these constructs. All the tissue-engineered vascular constructs displayed the general viscoelastic behavior indicative of most collagenous tissues. Constructs produced

with arterial cells were stronger and stiffer when compared with those produced using venous cells. Moreover, cyclic loading showed that tissue produced using arterial cells were able to bear higher loads for the same amount of strain when compared with vascular constructs produced with venous cells. This result could be attributable to the presence of elastin in aTEVM and aTEVMA, although further analysis such as transmission electron microscopy for the presence of mature elastic fibers and long-term fatigue testing would be necessary to fully correlate these results.[34] Taken together, these results indicate that arterial cells have the ability to produce vascular constructs that possess superior mechanical properties when compared with constructs produced using venous cells isolated from the same umbilical cord.
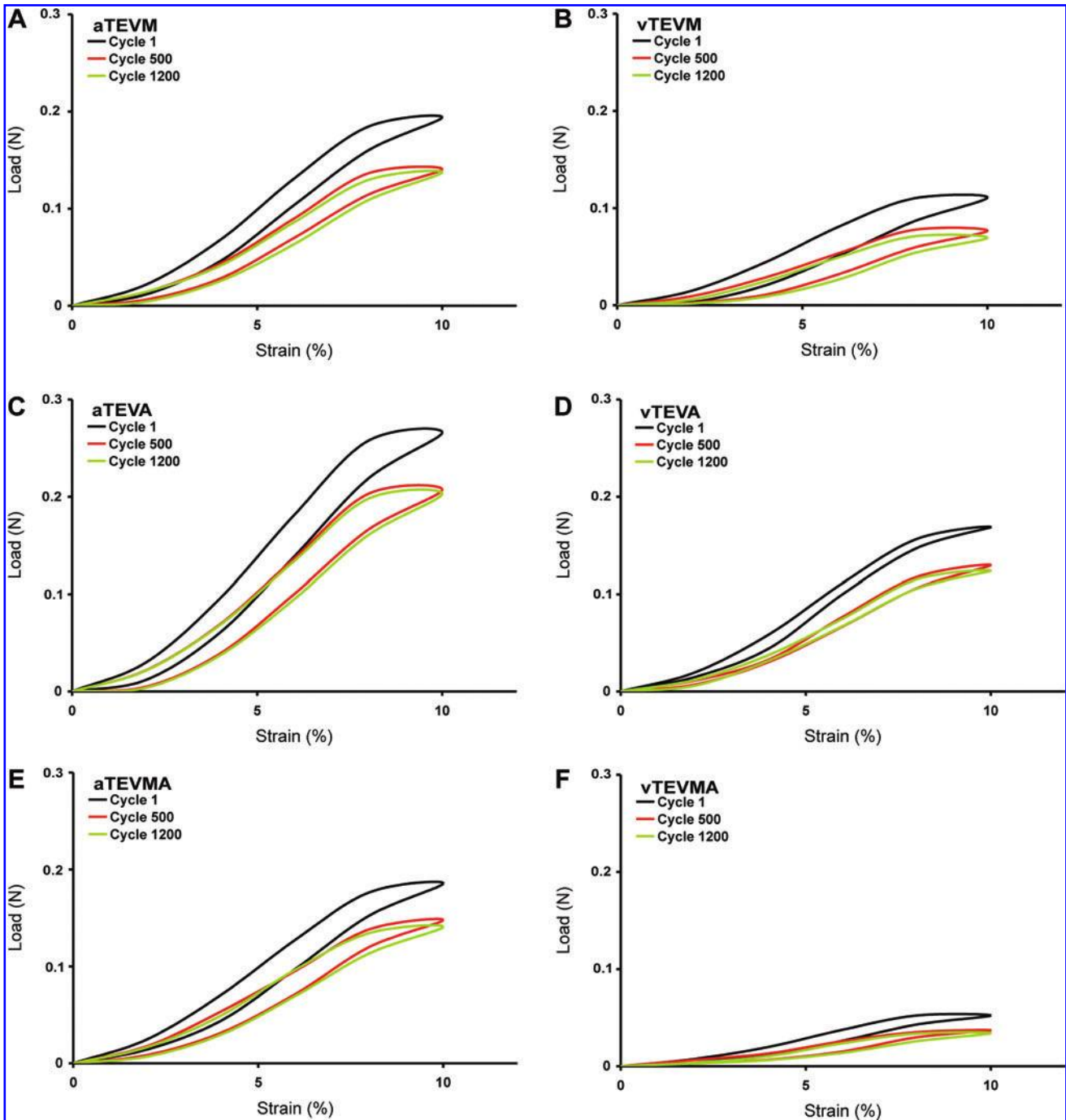
The umbilical cord is a potential cell and tissue source that can be used for the development of cardiovascular tissue engineering applications, as it contains both healthy arterial and venous cell types and it can be readily obtained. The applicability of cells isolated from the umbilical cord and cultured *in vitro* for vascular tissue fabrication was shown to

FIG. 3. Stepwise stress-relaxation data obtained for the arterial and venous tissue-engineered vascular constructs. Characteristic time-dependant stress-relaxation profile **(A)** showing the initial modulus (top line) and the equilibrium modulus (bottom line). Initial **(B)** and equilibrium **(C)** moduli showing that TEVMA displayed higher moduli in the case of vascular constructs produced using arterial cells. Modulus ratio **(D)** indicating whether the vascular constructs viscoelastic behavior is dominated by viscous or elastic attributes. Modulus subtraction **(E)** allowing for the comparison of the viscous and elastic components between the different conditions. Results are expressed as mean ± standard deviation. *Statistical significance within the same condition and **statistical significance in comparison to other conditions ($p < 0.05$). TEVMA, TEVM constructs comprised of both a media and an adventitia.

be comparable to the one of saphenous vein cells, which is a well-established cell source for vascular tissue engineering.[35] It was also recently proposed that the umbilical artery could be used as a decellularized scaffold for small-diameter vascular grafts, resulting in a patent tissue-engineered vascular conduit able to sustain physiological conditions up to 8 weeks after implantation into an animal model.[36] Cells extracted from the umbilical cord were also successfully used for scaffold seeding in the fabrication of tissue-engineered heart valves[37] and vascular conduits.[38,39] However, the im-

pact of using arterial versus venous cells for tissue engineering applications remained unclear. Previous work using adult cells, isolated either from human aortic tissue or from saphenous vein segments and then seeded on a polymer scaffold, showed that venous cells increased both collagen content and mechanical properties of these scaffolds when compared with aortic cell sources.[40] These findings differ from the results obtained in the current study, showing that under the tested conditions, arterial cells produce a stronger and stiffer tissue when compared with constructs produced

FIG. 4. Hysteresis curves obtained after continuous 10% strain loading and unloading cycles applied on the arterial **(A, C, E)** and venous **(B, D, F)** engineered vascular constructs. Both arterial and venous TEVM **(A, B)**, TEVA **(C, D)**, and TEVMA **(E, F)** displayed elastic attributes. Note that vascular constructs produced using arterial cells were able to withstand a higher load for the same amount of deformation at every cycle. All vascular constructs reached a state of equilibrium between the 500th and 1200th cycle. No condition experienced continuous creep in response to the application of the deformation cycles. TEVA, tissue-engineered vascular adventitia. Color images available online at www.liebertonline.com/tea

using venous cells, which is in accordance with the behavior of native vessels instating that arterial blood vessels display superior mechanical strength than venous blood vessels.[41] However, it is difficult to compare the outcome of studies performed using different cell types and tissue engineering technologies, especially if one involves the interaction of cells, often isolated from different species at different stages of development and displaying different phenotypes, with or

without a scaffold.[42] In the present study, the impact of complicated issues such as interpersonal variability and the age of each donor on the results were avoided by extracting all cell lines from the same cord and by using fully autologous cells for vascular construct production.

This method also shows great potential for pediatric applications, especially in the case of congenital heart disease surgeries requiring invasive treatment, where the amount of

available tissue is limited. Indeed, changing the diameter of the tubular support used for tissue production would allow for the production of a vascular construct having the appropriate diameter depending on the requirement of the pathology. Previous results have also shown that vascular constructs produced *in vitro* by self-assembly are vasoactive, therefore increasing their functionality and enhancing their physiological-like behavior.[43,44] Based on the literature and to improve the mechanical properties of the vascular constructs produced with umbilical cord cells, we are currently investigating the possibility of culturing these vascular constructs in a bioreactor under a physiologic environment to induce tissue remodeling and increase ECM production.[45] This could contribute to improve tissue functionality before implantation, therefore resulting in enhanced performance of self-assembled TEBV.[8,15,46–48] Other approaches involving microfabrication techniques and contact guidance principles are also under investigation to direct cell and ECM alignment.[49] These techniques have proved to be very reliable and to allow the engineering of tissues having anisotropic properties, therefore contributing to their biomimetic mechanical architecture.[50,51] Although essential to TEBV functionality, the endothelium was not added to vascular constructs used in the present study. It is well known that the patency of a small-diameter vascular graft depends on the presence of an endothelium at the blood-graft interface to avoid thrombus formation. Since ECs do not contribute significantly to the mechanical properties of blood vessels, we did not include the endothelial layer to the vascular constructs. The ECs participate in many ways to the shear stress and vasoactive signaling occurring in the vasculature and are known to influence tissue homeostasis and ECM regulation. Therefore, it would be important to include an endothelium in a subsequent study aimed at the evaluation of the complete TEBV functionality.

Conclusion

The potential of umbilical cord cells for vascular tissue engineering applications has already been shown in studies involving seeding of arterial and venous cells on a bioabsorbable polyglycolic-acid (PGA) scaffold.[35,38] Further, both the umbilical artery and vein have been previously used as decellularized scaffolds in combination with cell seeding techniques for vascular tissue engineering applications.[36,52] This study highlights the fact that cells isolated from arterial and venous umbilical tissue can be used to produce scaffold-free tissue-engineered vascular constructs. Mechanical properties of constructs produced using arterial cells were found to be superior to those obtained from constructs produced with venous cells. Further, higher level of elastin was detected in ECM produced by arterial SMCs compared with any other vascular cell types under the condition tested. Therefore, results show that self-assembled engineered tissues display cell-source and cell-type dependent differences, as strength, stiffness, and viscoelastic properties obtained were different for vascular constructs produced with arterial or venous SMCs and fibroblasts. The capability of producing fully autologous TEBV using cells isolated from the umbilical cord provides new insights in the search of an optimal cell source for vascular tissue engineering. Ultimately, this could also provide cells for multiple tissue engineering applications

without the need for harvesting of intact tissues, which present a significant advantage when comparing with other tissue engineering strategies.[53]

Disclosure Statement

No competing financial interests exist.

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