CELL DELIVERY IN TISSUE ENGINEERING

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Scaffolds used for cell delivery in tissue engineering should mimic the native structure of target tissues, degrade gradually with tissue formation, and coexist in a mechanically dynamic environment. The latter requirement implies that the scaffold should maintain its integrity and recover from various deformations without mechanical irritations to the surrounding tissues. In particular, we are interested in how scaffold mechanics and compartmentalized cell seeding can contribute to improved tissue neoformation. To study this phenomenon we have been developing a novel family of biodegradable polyester elastomers referred to as polydiol citrates, Yang et al., (2004). In addition we have been developing novel scaffold microarchitectures to enable compartmentalization of specialized cells in order to generate complex tissues such as blood vessels, ligament, and cartilage. Regarding cell delivery in vascular tissue engineering, the anatomy of a native blood vessel shows that endothelial cells are separated from surrounding smooth muscle cells by internal elastic lamina[G1]$_e$, which prompted us to fabricate a biphasic scaffold for blood vessel tissue engineering that would recapitulate such cell compartmentalization. As for orthopaedic tissue engineering, mechanical conditioning regimens, which have been shown to influence tissue neoformation (Kim, et al., (1994), Wong et al., (1999), Bonassar, et al., (2001)) would benefit from the use of a scaffold that can endure cyclic deformation and stress transfer during long culture times. Herein we describe the feasibility of using poly(diol citrates) as a cell delivery vehicle in vascular and orthopaedic tissue engineering. These materials have the following advantages: non–toxic monomers, relatively simple synthesis that can be carried out under mild conditions without addition of toxic catalysts or crosslinking reagents, controllable mechanical and biodegradation properties, easy processing, and inherent surface affinity for various cell types.

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MATERIALS AND METHODS

The following poly(diol citrates) were synthesized via a condensation reaction between citric acid and one of the following diols: 1,8–octanediol, 1,10–decanediol, and 1,12–dodecanediol. Details of the synthesis have been described elsewhere (Yang, et al., (2004)). The degradation characteristics of the various poly(diol citrate) films were investigated by measuring weight loss due to exposure to phosphate buffered saline (PBS). For vascular tissue engineering, the biphasic scaffold for small–diameter blood vessel application consists of a solid, continuous phase for endothelial cell attachment and monolayer formation within the vessel lumen and a surrounding porous phase with an inter–connective pore structure for smooth muscle cell growth and differentiation. In order to obtain a biphasic scaffold, a pre–polymer of the poly(diol citrate) was coated onto a glass rod and air–dried. The coated rod was then inserted in a cylindrical mold that contained a salt/pre–polymer mixture to create a porous outer phase. Poly(diol citrate) films and tubular scaffolds were subjected to various mechanical tests, including tensile and burst pressure. Human aortic endothelial (HAEC) and smooth muscle (HASMC) cells were seeded sequentially on the scaffold in batch mode under mild agitation and co–cultured for up to two weeks. For cartilage tissue engineering, a porous disc (2 mm thick by 5 mm diameter) was prepared by mixing the pre–polymer solution of poly(1,8–octanediol–co–citrate) (POC) with salt particles, crosslinking at 120^{o}C for three days, soaking in water for 96 hrs to leach out the salt, and stamping out the disc–shaped samples. The pore size data was obtained using image analysis software (Image–Pro® Plus V.4.0, Silver Spring, MD). The porosity of the scaffold was measured using a method based on Archimedes' principle as described elsewhere (Yang, et al, (2002)). To assess recovery from cyclic deformation, scaffolds were subjected to 500 cycles of compressive deformation using an Instron 5544 (Instron, Canton, MA). POC scaffolds were seeded in batch mode with bovine chondrocytes obtained from the femoral condyles and harvested after 2, 14, and 28 days of culture. Cell–seeded porous polyglycolide (PGA) scaffolds were used as controls. Scaffolds at the various

time points were analyzed for DNA, total collagen, and glycosaminoglycan (GAG) content. They were also analyzed using histology (cell and GAG distribution via Safranin–O, collagen via Masson's trichrome) and immunohistochemistry (collagen type II). Cell–scaffold constructs were assessed via scanning electron microscopy (SEM) and light microscopy.

RESULTS AND DISCUSSION

Poly(diol citrates) can exhibit in vitro degradation characteristics that may range from a time scale of months to years. Comparative degradation studies among the various poly(diol citrates) resulted in the following ranking according to increasing degradation rate: 1,8–octanediol, 1,10–decanediol, and 1,12–dodecanediol. The results suggest that the degradation rate can be modulated via the hydrophilicity of the diol that will be reacted with the citric acid monomer as the degree of hydrophilicity of the diols also follows the same order. A biphasic tubular scaffold that possessed an inner skin lining the lumen and a concentric outer porous phase was successfully produced. Scanning electron microscopy confirmed that the solid and porous phases are in fact connected. Burst pressures of 1300 mmHg could be achieved, a value that is comparable to that of veins. Ultimate tensile strength of poly(diol citrate) films ranged from 2 to 12 MPa and strains were as high as 300% initial length. Young's moduli ranged from 1.5 to 19 MPa. Vascular cells attached, proliferated and were compartmentalized within the biphasic scaffold. Regarding the use of poly(diol–citrates) for cartilage tissue engineering, POC porous scaffolds recovered completely from 500 cycles of deformation. Chondrocyte proliferation and matrix synthesis (as assessed by the markers used in this study) on POC scaffolds was comparable to that of chondrocytes seeded on PGA scaffolds. Cells attached to POC and maintained a rounded morphology as assessed via SEM.

CONCLUSIONS

Poly(diol–citrates) are biodegradable polyester elastomers whose degradation rate can be controlled with the choice of diol and condensation reaction conditions. The results suggest that a biphasic scaffold design that is based on poly(diol–co–citrates) is a viable strategy towards the engineering of small diameter blood vessels. Poly(1,8–octanediol–co–citrate) was conducive to neocartilage formation in vitro and the tissue formation was similar to that on PGA–based constructs.

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