Optimizing Tissue Scaffold Composition for X-ray-Based Imaging

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Introduction

X-ray imaging techniques can be very useful for tissue engineering applications. The ability to image radiopaque medical implants *in vivo* with X-ray radiography enables clinicians to conveniently, inexpensively and non-invasively monitor wound healing and regeneration *in situ.*[1,2] In addition, the internal microstructure of radiopaque implants can be observed *in vitro* with X-ray microcomputed tomography (μCT). However, highly porous tissue engineering scaffolds made from amorphous polymers such as PDLLA [poly(D,L-lactic acid)] or pDTEc [poly(desaminotyrosyl-tyrosine ethyl ester carbonate)][2] are essentially translucent to X-rays and thus, difficult to image with X-ray based techniques. One approach to make polymeric tissue scaffolds radiopaque is to incorporate high contrast atoms, such as iodine, into the polymer. However, molecular changes to the polymer often affect physicochemical properties and influence performance. Therefore, it may be necessary to determine the minimum amount of contrast agent necessary for imaging by X-ray techniques. In this study we have applied a new method for screening polymeric tissue scaffolds to determine the amount of radiocontrast agent required to successfully image a scaffold by X-ray radiography and μCT.

Methods

In order to enhance the radiopacity of pDTEc scaffolds, we have blended pDTEc (1.9 x 10^5 g/mol) with pI_2DTEc (2.9 x 10^5 g/mol). pI_2DTEc is an analog of pDTEc which is radiopaque due to iodination of the desaminotyrosine ring (Fig. 1).[2]

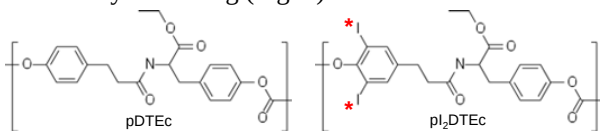

Fig. 1. Chemical structure of pDTEc (left) and pI_2DTEc (right).

A two-syringe pump system was designed to fabricate spatially-resolved compositional libraries of polymer scaffolds.[3] Briefly, solutions of pI_2DTEc and pDTEc (8.5 % by mass in dioxane) were placed in opposing syringe pumps, brought together at a T-junction and mixed in a static mixing tube. The pumps were programmed so that one *ramped up* from "off" to a 0.5 mL/min while the other pump *ramped down* from 0.5 mL/min to "off". In this way, the composition of the eluent from the static mixer changed from pI_2DTEc-rich to pDTEc-rich over time. The eluent from the static mixer was deposited into a rod-shaped trough 75 mm long that contained sieved NaCl crystals (0.25 mm to 0.425 mm dia.) and was mounted on a translation stage. The stage was programmed to move (3.75 cm/min) while the polymer solutions were deposited on the salt. Libraries were frozen, freeze-dried and salt leached in water. This process yielded rod-shaped scaffold libraries containing a gradient in composition from pI_2DTEc to pDTEc (Fig. 2).

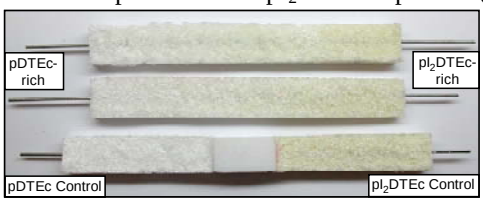

Fig. 2. Rod-shaped pDTEc/pI_2DTEc scaffold libraries are at top of image. Control pDTEc (left) and pI_2DTEc (right) scaffolds are at bottom. Stainless steel wires were incorporated into the scaffolds for reinforcement and to ease handling. Gradients range from 5 % to 83 % pDTEc by mass.

The pDTEc/pI_2DTEc scaffold libraries were analyzed with X-ray radiography (HP Cabinet X-ray System, Faxitron Series; Settings: 80 kVp, 3 mA, 3 min exposure) and X-ray μCT (μCT-40, Scanco Medical; Settings: 45 kVp, 177 μA, 300 ms integration time, average data 2, 8 μm resolution) to determine the amount of pI_2DTEc required for optimal imaging.

Results/Discussion

X-ray μCT scans of pure pDTEc and pI_2DTEc control scaffolds (Fig. 3) showed that pI_2DTEc yields a sharp image that can be quantitatively analyzed. The pDTEc scaffold yields a noisy image where pores are not well-defined. Analysis of gradient libraries showed that scaffolds with at least 20 % by mass pI_2DTEc were clearly visible in X-ray radiographs (not shown) while scaffolds with at least 50 % by mass pI_2DTEc were required for optimal imaging by X-ray μCT (not shown).

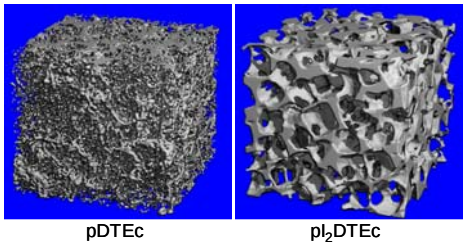

Fig. 3. X-ray μCT images of control pDTEc (left) and pI_2DTEc (right) scaffolds. Images are 2 mm cubes.

Conclusions

These results describe a new approach for determining the minimum amount of X-ray contrast agent required for effective imaging of polymer scaffolds by X-ray radiography and μCT.

References
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3. Simon Jr CG, Stephens JS, Becker ML. Submitted;2007.