

## 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*.<sup>1,2</sup> In addition, the internal microstructure of radiopaque implants can be observed *in vitro* with X-ray microcomputed tomography ( $\mu$ CT). However, highly porous tissue engineering scaffolds made from amorphous polymers such as PDLA [poly(D,L-lactic acid)] or pDTEc [poly(desaminotyrosyl-tyrosine ethyl ester carbonate)]<sup>2</sup> 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  $\mu$ CT.

### Methods

In order to enhance the radiopacity of pDTEc scaffolds, we have blended pDTEc ( $1.9 \times 10^5$  g/mol) with pI<sub>2</sub>DTEc ( $2.9 \times 10^5$  g/mol). pI<sub>2</sub>DTEc is an analog of pDTEc which is radiopaque due to iodination of the desaminotyrosine ring (Fig. 1).<sup>2</sup>

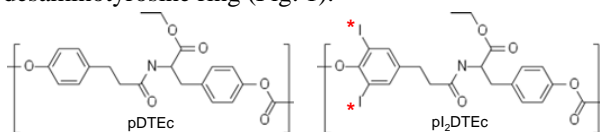


Fig. 1. Chemical structure of pDTEc (left) and pI<sub>2</sub>DTEc (right).

A two-syringe pump system was designed to fabricate spatially-resolved compositional libraries of polymer scaffolds.<sup>3</sup> Briefly, solutions of pI<sub>2</sub>DTEc 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<sub>2</sub>DTEc-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<sub>2</sub>DTEc to pDTEc (Fig. 2).



Fig. 2. Rod-shaped pDTEc/pI<sub>2</sub>DTEc scaffold libraries are at top of image. Control pDTEc (left) and pI<sub>2</sub>DTEc (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<sub>2</sub>DTEc 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  $\mu$ CT ( $\mu$ CT-40, Scanco Medical; Settings: 45 kVp, 177  $\mu$ A, 300 ms integration time, average data 2, 8  $\mu$ m resolution) to determine the amount of pI<sub>2</sub>DTEc required for optimal imaging.

### Results/Discussion

X-ray  $\mu$ CT scans of pure pDTEc and pI<sub>2</sub>DTEc control scaffolds (Fig. 3) showed that pI<sub>2</sub>DTEc 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<sub>2</sub>DTEc were clearly visible in X-ray radiographs (not shown) while scaffolds with at least 50 % by mass pI<sub>2</sub>DTEc were required for optimal imaging by X-ray  $\mu$ CT (not shown).

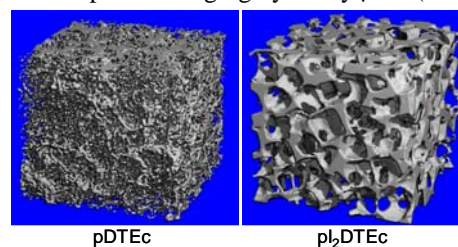


Fig. 3. X-ray  $\mu$ CT images of control pDTEc (left) and pI<sub>2</sub>DTEc (right) scaffolds. Images are 2 mm cubes.

### 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  $\mu$ CT.

### References

1. Kruft MA, van der Veen FH, Koole LH. *Biomaterials* 18, 31-36;1997.
2. Kohn JB, Bolikal D, Pendharkar SM. US Patent 7,056,493;2006.
3. Simon Jr CG, Stephens JS, Becker ML. Submitted;2007.

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