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X-ray imaging optimization of 3D tissue engineering scaffolds via combinatorial fabrication methods

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Abstract

We have developed a combinatorial method for determining optimum tissue scaffold composition for several X-ray imaging techniques. X-ray radiography and X-ray microcomputed tomography enable non-invasive imaging of implants *in vivo* and *in vitro*. However, highly porous polymeric scaffolds do not always possess sufficient X-ray contrast and are therefore difficult to image with X-ray-based techniques. Incorporation of high radiocontrast atoms, such as iodine, into the polymer structure improves X-ray radiopacity but also affects physicochemical properties and material performance. Thus, we have developed a combinatorial library approach to efficiently determine the minimum amount of contrast agent necessary for X-ray-based imaging. The combinatorial approach is demonstrated in a polymer blend scaffold system where X-ray imaging of poly(desaminotyrosyl-tyrosine ethyl ester carbonate) (pDTEc) scaffolds is improved through a controlled composition variation with an iodinated-pDTEc analog (pI_2DTEc). The results show that pDTEc scaffolds must include at least 9%, 16%, 38% or 46% pI_2DTEc (by mass) to enable effective imaging by microradiography, dental radiography, dental radiography through 0.75 cm of muscle tissue or microcomputed tomography, respectively. Only two scaffold libraries were required to determine these minimum pI_2DTEc percentages required for X-ray imaging, which demonstrates the efficiency of this new combinatorial approach for optimizing scaffold formulations.

Keywords: Combinatorial library; Polycarbonate; Scaffolds; Radiopacity; X-ray microcomputed tomography; X-ray radiography

1. Introduction

The ability to image radiopaque medical implants *in vivo* with X-ray radiography enables clinicians to conveniently, inexpensively and non-invasively monitor implant performance, wound healing and regeneration. In addition, the internal microstructure of radiopaque implants can be analyzed *in vitro* with X-ray microcomputed tomography (μCT). However, highly porous tissue engineering scaffolds made from polymers such as PDLLA [poly(D,L-lactic acid)] or pDTEc [poly(desaminotyrosyl-tyrosine ethyl ester carbonate)] do not possess high X-ray contrast and can yield poorly resolved images. The radiopacity of conventional polymers is similar to soft tissue since both are composed primarily of hydrogen, oxygen, nitrogen and carbon. Material X-ray contrast can be improved by increasing material electron density through addition of heavy atoms such as barium, bismuth or iodine [1].

Approaches for adding heavy atoms include: (1) physical mixture of salts, such as addition of barium sulfate to poly(methylmethacrylate) (PMMA) [2] or poly(D,L-lactic acid) [3], (2) blends with organic compounds, such as blending triphenyl bismuth with PMMA [4], and (3) covalent linkage of heavy atoms to the polymer backbone [5], such as iodinating pDTEc to yield pI_2DTEc [6,7]. Each approach has its own advantages and disadvantages (reviewed in Ref. [1]). One common problem is that the inclusion of radiocontrast agents will affect

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physicochemical properties, and, thus, performance of the implant [1–4]. In order to minimize physicochemical changes, it would be advantageous to determine the minimum amount of radiocontrast agent required to impart radiopacity to the implant. Thus, we have developed a combinatorial approach for screening polymeric tissue scaffolds to determine the amount of radiocontrast agent required for effective imaging by common X-ray techniques.

Combinatorial methods can lower the cost of experimentation through fabrication of miniaturized libraries which enable accelerated testing of many specimens and reduce the amount of time and material required for experiments [8]. They have been applied extensively in pharmaceutical research [9,10] and their application in biomaterials research is also becoming widespread [11–15]. The combinatorial approach developed herein is designed for characterizing and optimizing the radiopacity of tissue engineering scaffolds since these constructs constitute a central dogma in the field of tissue engineering [16]. The basic premise of a tissue scaffold is a highly porous, degradable, biocompatible construct that promotes the formation of a desirable tissue by providing a 3D template for cell adhesion, differentiation and tissue generation [17].

A tyrosine-derived polycarbonate polymer system was used to demonstrate the combinatorial approach for screening scaffold radiopacity. Tyrosine-derived polycarbonates are a biodegradable, biocompatible class of polymers being developed for tissue engineering applications [6,15,18]. For the current study, the radiopacity of pDTEc was enhanced through a controlled blending with its iodinated analog, pI_2DTEc (Fig. 1a,b). Although the toxicity of an iodinated, resorbable biomaterial is a concern, preliminary results injecting iodinated, resorbable polyesters into rabbits for imaging by computed tomography showed no adverse affects [5]. In addition, a fully resorbable coronary stent made from a pI_2DTEc derivative is currently in human clinical trials (Reva Medical, Inc., San Diego, CA, http://www.teamreva.com). Thus degradable, radiopaque polymers such as pI_2DTEc are being considered for use as implantable biomaterials. A two-syringe pump system was used to fabricate spatially resolved compositional scaffold libraries of pDTEc and pI_2DTEc [19], the libraries were imaged by several common X-ray imaging techniques and images were analyzed to determine the minimum amount of pI_2DTEc required for effective imaging. These results demonstrate how combinatorial methods can efficiently identify scaffold formulations that contain the minimum amount of radiocontrast agent necessary for effective imaging by X-ray-based techniques.

2. Materials and methods

2.1. Gradient scaffold library fabrication

Combinatorial scaffold libraries were fabricated from pDTEc (185,000 weight averaged molecular weight (M_w); 1.8 polydispersity index (PDI)) and pI_2DTEc (294,000 M_w; 1.8 PDI) (Fig. 1a,b) synthesized as described [6,18] using a novel syringe-pump system (Fig. 1c) [19]. For library fabrication, solutions of pDTEc and pI_2DTEc (1 g/10 mL dioxane) were placed in opposing syringe pumps, brought together at a T-junction and mixed in a static mixer. The pumps were programmed so that the effluent from the static mixer changed from pI_2DTEc-rich to pDTEc-rich over time. The effluent from the mixer was deposited into a teflon trough (75 mm long × 8 mm wide × 6 mm deep) containing 4.3 g of sieved NaCl (250–425 μm in dia.). A stainless steel wire was incorporated lengthwise into the troughs to facilitate handling. A motorized stage was used to translate the trough during deposition of the polymer solutions to create a composition gradient that went from pI_2DTEc-rich to pDTEc-rich.

After the deposition, libraries were frozen in liquid nitrogen, freeze-dried overnight to remove solvent, leached in water for 4 days to remove NaCl, air dried and stored in a desiccator until use. Control scaffolds of pure pDTEc and pure pI_2DTEc were fabricated using only one syringe pump and the translation stage. A control poly(D,L-lactic acid) (PDLLA, (330,000–600,000) M_w, Polysciences, Warrington, PA) scaffold was also fabricated for comparison. Only two pDTEc/pI_2DTEc libraries plus controls were used for this entire study (Fig. 1d, PDLLA control not shown). Each pDTEc/pI_2DTEc library weighed 85 mg and approximately 500 mg of polymers were used to fabricate the two libraries (extra polymer solution is used for mixing and priming lines). As for experimental logistics, both libraries were first imaged by the three X-ray techniques (microradiography, dental radiography, μCT). Next, one library was used for scanning electron microscopy (SEM) and the second library was used for Fourier transform infrared spectroscopy (FTIR).

2.2. Fourier transform infrared spectroscopy

Composition of the combinatorial polymer scaffold libraries was characterized using FTIR (NEXUS 670 FTIR spectrophotometer, Nicolet, Thermo Electron, Madison, WI). A library was cut into 10 sections of length 7.5 mm and dissolved in chloroform. Measurements were conducted by casting the polymer solutions onto a KBr pellet and recording spectra at a resolution of 4 cm^{-1}, 64 scans and total range 4000–650 cm^{-1}. Analysis was performed with OMNIC (Version 7.2, Thermo Electron). Baseline deduction and normalization to maximum absorbance were performed on all spectra. A calibration curve was established using FTIR spectra of nine blends of known pDTEc/pI_2DTEc composition (Fig. 2b inset). Absorbance at 2935 cm^{-1} was chosen as the reference band (methyl stretching) while absorbance at 710 cm^{-1} was chosen as the analytical band (*ortho*-phenyl ring substitution, iodine) [18,20]. Peak height ratios of sections (710 cm^{-1}/2935 cm^{-1}) from the combinatorial scaffold libraries were determined and corresponding compositions were calculated using the calibration curve [21]. Peak height was calculated from baseline.

2.3. Scanning electron microscopy

Scaffolds were frozen in liquid nitrogen and sectioned with a razor to expose interior. After sputter-coating with gold, pores were viewed by SEM (15 kV, Hitachi S-4700-II FE-SEM, Pleasanton, CA). ImageJ (1.37v, Wayne Rasband, National Institutes of Health, USA) was used to measure diameter of six randomly chosen pores in SEM images at the compositions indicated.

2.4. X-ray microradiography and dental radiography

Scaffold libraries plus controls were imaged in an X-ray microradiometer (80 kVp, 3 mA, 180 s exposure, closest possible distance setting, HP Cabinet X-ray System, Faxitron Series, McMinnville, OR) using X-ray film (VRP-M green sensitive film, UAB Geola, Vilnius, Lithuania) and with a dental X-ray instrument (70 kVp, 7 mA, 0.13 s exposure (eight pulses), 75 mm distance, Gendex GX-770, Lake Zurich, IL) using 75 mm occlusal film (DF-50 single, Ultraspeed, Kodak, Rochester, NY). Developed X-ray films were digitally imaged (VersaDoc, Bio-Rad, Hercules, CA) for presentation in figures and for densitometry analysis using Quantity One software (Bio-Rad). Ten evenly spaced regions 7.5 mm in length running lengthwise along each gradient library were selected for densitometry. Background regions were subtracted from libraries and controls. Note that OD values for samples with high X-ray contrast, such as control pI_2DTEc scaffolds, become negative after background subtraction. This is because radiographs are "negatives" and objects with high X-ray contrast appear light and have low pixel intensity values while background areas are dark and have high pixel intensity values.