INVESTIGATION OF 3-D TYROSINE-DERIVED POLYCARBONATE SCAFFOLDS FOR TISSUE ENGINEERING

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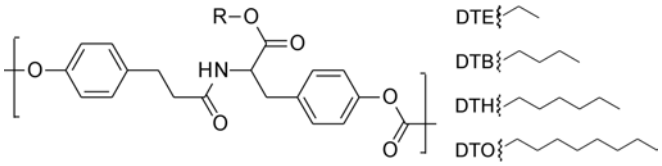
Introduction

A library of tyrosine-derived polycarbonates have been developed that share a structurally identical backbone with a rich chemical and structural diversity, which depend solely on the pendent ester substituent (R) (Scheme 1).[1] The physical and chemical nature of the pendant group significantly affects the mechanical properties, degradation rates, and cellular responses of each polymer.[2] This study will explore the effect of discrete blends of the tyrosine-derived polycarbonates in tissue engineering scaffolds by investigating materials properties and cell/materials interactions.



Scheme 1. General chemistry of tyrosine-derived polycarbonates and side chains.

Results and Discussion

A valid criticism of *in vitro* and high throughput assays is that they may not accurately predict the *in vivo* response. Our efforts are to develop and compare all scaffold types (thin film discrete blends and gradients and 3D scaffolds under both static and dynamic culturing conditions) and at each step to find potential differences and explore the nature of the responses found in each case. Once we have made the measurements at each step on well-characterized materials, we anticipate learning about the processes and tolerance thresholds for drawing comparisons between data collected from each scaffold type.

The first stage of our study investigated these homopolymers and blends.[3] Figure 1 shows thin films of tyrosine-derived polycarbonate DTE and DTO and discrete blends of the two. From this we have begun to identify the surface chemistry, energy and roughness that results from annealing and phase separation of the film. These findings have also been correlated to cellular response (inflammatory response and extracellular matrix (ECM) production) to the films. The next phase is to investigate these homopolymers and blends in three dimensional (3D) tissue engineering scaffolds for bone regeneration. Figure 2 compares salt leached tyrosine-derived polycarbonate DTE scaffolds, illustrating that there is no visual change in the scaffold structure as a result of annealing. The ability to explore the 3D interconnected porous nature of the scaffolds, to truly compare the influence of processing conditions on the structural features, would be advantageous. Another challenge that faces researchers is the ability to explore the 3D interconnected porous nature of tissue engineering scaffold and how it relates to cell/cell and cell/scaffold interactions.

Figure 1. From left to right are five 5 μm × 5 μm AFM images in which the amount of DTO is increasing. The discrete DTE/DTO blends form phase-separated domains under *in vacuo*, 105 °C annealing conditions.

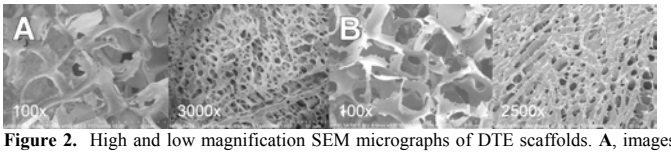


Figure 2. High and low magnification SEM micrographs of DTE scaffolds. **A**, images of the as leached unprocessed DTE scaffold and **B** images of a scaffold from the same batch that had been annealed with the salt *in vacuo* at 105 °C for 16 h followed by a dialysis step to remove the salt.

At NIST we have been developing an optical imaging system to nondestructively and noninvasively investigate the structure and function of polymeric tissue engineering scaffolds. Collinear optical coherence/confocal fluorescence microscopy (OCM/CFM) allows for high spatial resolution and good depth of field through highly scattering polymeric materials.[4,5] OCM uses interferometry rather than a pinhole to reject out-of-focus light and is 6 to 7 orders of magnitude more sensitive than traditional confocal microscopy, which translates into deeper imaging depth. This method provides a means of non-destructive, high resolution, *in-situ* imaging to gather structural information of a 3D scaffold. The tyrosine-derived DTE homopolymer scaffold has been examined using OCM. Figure 3 depicts a wire frame reconstruction of a DTE scaffold immersed in water using OCM. The scaffold is rendered in green wire frame while the pores are in black. Of significance are the 400-500 μm depths, which are measurable using this technique. Initial results show that the DTE scaffold is highly interconnected.

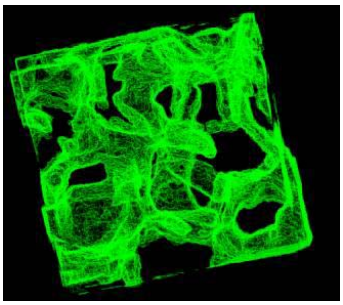


Figure 3. OCM of DTE scaffold, dimensions 1 mm x 1mm x 400 μm.

Conclusions

The results of the discrete blends work in the 2D films have laid a strong foundation for the work we will explore in the scaffolds. The goals of this work is to continue to explore the structural features (pore size, pore interconnectivity) of the scaffolds and the corresponding cell interactions using OCM/CFM and conventional assays in order to determine if a combination of polymer properties increases bone activity.

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[+]Non-statistical data.

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