Gene Expression Profiles of Cells in Response to Tyrosine Polycarbonate Blends

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Introduction
A series of tyrosine-derived polycarbonates have been reported as promising degradable polymers for use in orthopedic, tissue engineering and drug delivery applications.¹-⁴ The physical and chemical nature of the pendant ester substituent affects significantly the mechanical properties, degradation rates, and cellular responses. Esters of the tyrosine-derived polycarbonates corresponding to the ethyl, butyl, hexyl, and octyl side chain alkyl groups have been prepared for use in the studies described herein and are denoted DTE, DTB, DTH, and DTO respectively in Scheme 1. The characterization data for the respective polymers, which differ structurally only by the length of the alkyl side chain group, are listed on the right.

Scheme 1. Structural and Characterization Data for Tyrosine Derived Polycarbonates

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mₘ (×10⁴)</th>
<th>Tg (°C)</th>
<th>Contact angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTE</td>
<td>131</td>
<td>98</td>
<td>73</td>
</tr>
<tr>
<td>DTB</td>
<td>79.1</td>
<td>72</td>
<td>77</td>
</tr>
<tr>
<td>DTH</td>
<td>57.3</td>
<td>62</td>
<td>86</td>
</tr>
<tr>
<td>DTO</td>
<td>61.6</td>
<td>51</td>
<td>90</td>
</tr>
</tbody>
</table>

Most materials implanted in hard tissue tend to grow a fibrous capsule at the bone-implant interface. Capsule formation is usually indicative of a mild inflammatory response. For the tyrosine-based polycarbonates, small changes in alkane chain length to the pendant ester group caused significant changes in the observed capsule formation and resulting clinical outcome, which indicated that the extent of calcium deposition on the biomaterial was a key parameter in determining bone adhesion. Fundamental differences have been measured at the bone-implant interface for tyrosine derived polycarbonates.⁵ For example, DTE produced nearly 70 % apposition and 30 % encapsulation, while DTO showed less than 20 % apposition.⁵ Additional studies have demonstrated a linear correlation between cell proliferation and air-water contact angles for polymers possessing an identical backbone structure but different pendant chains.⁶ Cell proliferation on polymers having no oxygen atoms decreased significantly as the polymer surface became more hydrophobic. The results of the numerous studies have concluded that of the four materials tested, DTE is the best performing polycarbonate in vivo. Our efforts to develop in vitro assays for the measurement of cytokine regulation and extracellular matrix (ECM) production and cellular apoptosis hinge on their ability to accurately reflect the known in vivo material performance are described. These efforts will be described along with additional work on the synthesis, characterization, and response of polycarbonate blends.

Results and Discussion
Inflammatory responses play a prominent role in the biocompatibility of materials, as indicated by the induction of the cytokines interleukin-1 beta (IL-1β) and tumor necrosis factor- alpha (TNF-α). Previously, real-time polymerase chain reaction (RT-PCR) measurements have quantitatively assessed the genetic expression profiles for these cytokines in response to tissue-engineered scaffolds,⁷ copolymer blends, and functionalized nanoparticles.⁸ In addition, cellular adhesion is essential for providing physical support and positional information for multicellular organisms.
The ECM has recently received considerable attention due to its importance in cell-cell signaling, wound repair, cell adhesion and tissue function.9

Previous studies looking at cytokine and extracellular matrix production of the respective homopolymers and tissue culture polystyrene (TCPS) depicted measurable differences in IL1-β production between the tyrosine-derived polycarbonates, ε-polycaprolactone (PCL), and TCPS. PCL and all of the tyrosine polycarbonates except for DTO exhibited significant increases (50- fold to 172- fold) in IL1-β production at 24 h. Additionally, we measured differences in the genetic expression of actin, fibronectin, and collagen I for this series of tyrosine-derived polycarbonates, PCL and TCPS. In the case of fibronectin, minimal differences were seen between the homo-polymers except for a general trend that was down regulation from the expression level relative to TCPS.

Figure 1 depicts the differences in actin, fibronectin and collagen I gene copy numbers for each of the tyrosine derived polycarbonates, (DTE, DTB, DTH, and DTO, respectively), TCPS, and PCL after 3 h of incubation at which time the vast majority of the bone osteoblasts were observed to be adhered to the substrates. Error bars are representative of one standard deviation from the mean of triplicate samples harvested from a single population of cells, and are the estimate of the standard uncertainties.

Significant differences in inflammatory cytokine expression and minimal changes in extracellular matrix production led us to believe that optimizations could be made with regard to composition. Preliminary studies described herein characterize a series of discrete blends of DTE and DTO and show differences in both IL-1β and fibronectin genetic expression. The expression levels are significantly different from both the homo-polymers and the respective blends. The ethyl, DTE, and octyl, DTO, tyrosyl esters were chosen because they possessed the largest differences in water contact angle and glass transition temperature and significant biological data has already been acquired for these materials. A series of blends were made to outline small areas of physical parameter space to probe whether significant differences in cell response could be measured. The series consisted of 30/70, 50/50, and 70/30 (by mass) DTE/DTO ratios.

Figure 2 depicts gene copy numbers of interleukin-1β (IL-1β, A & C) and fibronectin (B & D) after 24 h of surface exposure on the respective homopolymers and blends for RAW 264.7 macrophages (A & B) and MC 3T3 bone osteoblasts (C & D). Error bars are representative of one standard deviation from the mean of triplicate samples harvested from a single population of cells, and are the estimate of the standard uncertainties.

The results measured in these discrete samples highlight the need for high-throughput metrologies for the rapid and systematic evaluation of a candidate’s potential biocompatibility. NIST has developed several platforms for characterizing the physical properties of sample libraries with orthogonal gradients in thickness,10,11 composition,12 and morphology13 using combinatorial methodologies. These methods are being used to
create 1-D and 2-D gradients of DTE and DTO to examine further the optimal composition and processing conditions that maximize extracellular matrix production and minimize interleukin-1β response.

Conclusions

The evaluation and identification of detrimental interactions between biological species and synthetic surfaces is a daunting challenge as the number of materials and control of physical variables increases. In this talk, the physical properties of the homo-polymers and blends will be discussed in detail. In addition, recent RT-PCR results examining the expression profiles of IL-1β and fibronectin will be described.

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Disclaimer. Certain commercial materials and equipment are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation by NIST nor does it imply that the material or equipment identified is necessarily the best available for this purpose.

References