Synthesis of Novel Two-component Injectable Polyurethane Composites and Preliminary Assessment using Primary Human Osteoblasts

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Statement of Purpose: The recent development of injectable, biodegradable, and in-situ crosslinkable biomaterials seeks to alleviate many of the challenges associated with current surgical techniques and prefabricated tissue engineered implants (1,2). Injectable synthetic polymer based systems have the advantage of arthroscopic use in which a gel can be introduced into a defect with complex geometries to provide a strong bond with surrounding tissues. Mechanical properties, degradation times, and functionality can be easily tailored to support the tissue type, providing issues such as biocompatibility, sterilization, and ease of handling are addressed (1). A key aim is therefore to select not only biocompatible but also versatile polymers when designing these injectable in-situ forming polymer platforms. A new generation of lysine-diisocynate (LDI)-based polyurethane compositions possess the versatility of polyurethanes, but lack the toxicology of other urethane products and can also incorporate functionally active moieties to promote cell adhesion, viability, and proliferation (3). This study seeks to evaluate urethane-based novel two-component injectable and in-situ curable (ISC) polymers developed by PolyNovo Biomaterials (Melbourne, Australia), for orthopedic applications. The Novosorb™ polymer platform aims to: cure into a three dimensional structure with good initial mechanical properties and minimal heat generation, provide a biocompatible and non-toxic environment with non-immunogenic degradation products upon erosion, biodegrade in a time frame that complements new tissue ingrowth and regeneration, and provide support for cell adhesion and promote cell viability and proliferation. Here we have performed the initial characterization of the surface properties of the synthesized polymers, and evaluated the biocompatibility and cell-surface interactions with primary human osteoblasts

Methods: Four NovoSorb™ ISC polyurethane films (denoted 76-2, 76-2 TCP, 76-6, 76-6a) were prepared by reacting two prepolymers A and B in the presence of stannous octoate catalyst (0.1 wt %). In all samples prepolymer B was based on pentaerythritol (PE) and glycolic acid. In 76-2 samples prepolymer A was prepared from PE and ethyl, lysine diisocyanate (ELDI), whereas for the other two A was based on PE and l-lactic acid (76-6) or d,l-lactic acid (76-6a) end-capped with ELDI. In the case of sample 76-2 TCP, β-TCP was incorporated as a filler during synthesis. Dynamic contact angle analysis was performed to analyze surface wettability and roughness. Scanning electron microscopy coupled with energy dispersive X-ray (SEM/EDX) analysis was employed to examine surface topography and determine chemical composition of film surfaces properties. Films were seeded with primary human osteoblasts isolated from femoral heads and cultured for periods of up to 7 days, to study biocompatibility and cell behavior using Live/Dead and MTS assays.

Results / Discussion: Dynamic contact angle results reveal that all sample surfaces can be characterized as hydrophilic, with samples 76-2 and 76-2 TCP being the most hydrophilic. Analysis of contact angle hysteresis revealed that all samples have enhanced surface heterogeneities and roughness as compared to appropriate controls of thermanox and prefabricated PDLLA and polyurethane films. Further analysis of these surfaces heterogeneities on samples 76-2 and 76-2 TCP using SEM/EDX revealed correlations between surface topography and chemical content. Sample 76-2 TCP, a surface shown to have comparatively more surface heterogeneities, was found to contain local concentrations of calcium and phosphorus ions in these 'rough' areas. Flat, homogeneous areas, in contrast, were found not to contain concentrations of calcium and phosphorus.

Qualitative Live/dead analysis of cell viability revealed extremely positive live to dead cell ratios (>95%), indicating that the films were non-cytotoxic to primary human osteoblasts and maintained cell viability for periods of up to 7 days (see Fig 1.)

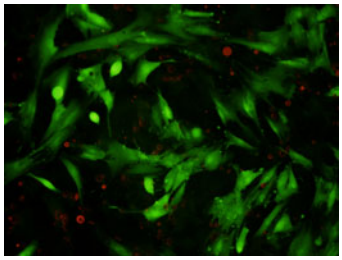

Figure 1: Live/Dead image of cells on sample 76-2 TCP

MTS results show that all films exhibit an increase in cell proliferation over a period of 7 days. This demonstrates that the films support normal metabolic function of primary osteoblasts for a period of 7 days. Sample 76-2 TCP was comparable (no significant difference) to cells on thermanox over the entire period of 7 days ($p<0.05$).

Conclusions: In this study, newly developed polyurethane based, injectable and in-situ curable polymer platform was evaluated as a potential material for tissue engineering. Based on preliminary characterizations of surface and biological properties, using various analytical techniques and primary osteoblasts, this Novosorb™ injectable polymer platform has a number of potential uses in orthopedic therapies, particularly as bone glues.

References:

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