Two Photon Induced Polymerization of Organic/Inorganic Hybrid Biomaterials For Microstructured Medical Devices

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Statement of Purpose:
A novel material and a novel processing technique were studied for fabricating microstructured medical devices. Two photon induced polymerization used typically for fabricating photonic structures and other microelectronic devices [1-3] was explored for applications in the biomedical field. Three dimensional tissue engineering scaffolds and microneedles were fabricated from Ormocer®, an amorphous organic-inorganic hybrid material that contains organically modified silicon alkoxides, metal alkoxides, and organic monomers. Femtosecond laser pulses from a Ti: sapphire laser were used with Irgacure® 369 photoinitiator within a small focal volume to polymerize the Ormocer® liquid. Ormocer® structures fabricated using two photon induced polymerization were tested for mechanical properties, cell-interaction, attachment and proliferation, and flow/fracture studies for microneedles. Lego®-like interlocking tissue engineering scaffolds and microneedle arrays with unique geometries were created using two photon induced polymerization.

Methods:
Three-dimensional microneedle arrays and tissue engineering scaffolds on glass substrates were fabricated using two photon induced polymerization of Ormocomp® (Ormocer® US-S4) and 1% Irgacure® 369 photoinitiator. Ormocomp® is a UV sensitive hybrid material that exhibits a refractive index at 589 nm of 1.520. The Irgacure® 369 initiator is frequently employed in Ormocer® device fabrication [1-3], and has an absorption peak at around 320 nm. Femtosecond laser pulses (60 fs) from a titanium: sapphire laser (94 MHz, < 450mW, 780 nm) are focused using a 20 x conventional plan achromat microscope objective into a small focal volume within a photosensitive resin. Cell growth and viability studies were performed using B35 neuroblast-like cells and HT1080 epithelial-like cells. B35 and HT1080 cells (ATCC, Manassas, VA) were sub-cultured in culture medium (DMEM (containing 4.5 g/L glucose, 110 mg/L sodium pyruvate, and 2 mM L-glutamine) supplemented with 10% (v/v) heat inactivated fetal bovine serum and 2 mM of L-glutamine). Fluorescent-viable cells were then examined with a LSM 510 confocal microscope (Carl Zeiss, Oberkochen, Germany).

Results / Discussion:
X-ray diffraction was used to confirm that Ormocer® surfaces created using two photon induced polymerization demonstrate amorphous behavior. The nanohardness and Young's modulus values for the polymerized Ormocer® were determined. Young's moduli values for Ormocer® materials may be varied between 1 MPa and 8 GPa through the use of various types of multi(meth)acrylate alkoxysilane precursors. Microneedle arrays for drug delivery were fabricated using two photon induced polymerization. Microneedles provide a unique approach for transdermal drug delivery of advanced DNA- and protein-based medicines. These microneedles exhibit 200 micrometer base diameters, and taper linearly to sharp tips over a needle length of 750 micrometer. The ten by ten microneedle arrays exhibits needle-to-needle spacing of 1 mm. These hollow microneedles demonstrate much larger wall thicknesses than those observed in microneedles created using conventional microfabrication techniques. Live/dead assays were performed to determine the viability of epithelial-like and neuroblast-like cells on Ormocer® substrates. The number of live epithelial-like and neuroblast-like cells was found to gradually increase over time. 100% of the B35 neuroblast-like cells and HT1080 epithelial-like cells remained viable 48 hours after innoculation on the Ormocer® substrates. These results suggest that Ormocer® does not significantly impair cell viability and cell growth. Two photon induced polymerization was used to develop Lego®-like interlocking tissue engineering scaffolds, which contain arrays of cylindrical pillars. These structures were created by fabricating arrays of cylindrical pillars on both sides of a flat Ormocer® chip. These structures could be used either as free-standing scaffolds or stackable blocks for layer-by-layer replication of heterogeneous tissues.

Conclusions:
Three dimensional Lego®-like interlocking tissue engineering scaffolds and microneedles were fabricated from Ormocer® hybrid materials using Irgacure® 369 photoinitiator. Ormocer® surfaces fabricated using two photon induced polymerization demonstrated acceptable cell viability and cell growth profiles against B35 neuroblast-like cells and HT1080 epithelial-like cells. The cell adhesion, cell viability, and cell growth behavior of B35 neuroblast-like cells grown on Lego®-like suggest that Ormocer® materials may provide a unique alternative to conventional polymeric scaffolds for creating multilayered artificial tissues. The results suggest that two photon induced polymerization is able to create medical microdevices with a larger range of sizes, shapes, and materials than conventional polymer, silicon, stainless steel, or titanium microfabrication techniques.

References:
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2. Houbertz R. et al, Adv Eng Mat 2003; 5: 551-55.
3. Serbin J. et al, Opt Express 2004; 12: 5221-5228.