Spatial patterning of structural properties in a photodegradable PEG-based hydrogel for cell culture

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Statement of Purpose: Studies have shown the importance of scaffold structural properties on cell behavior[1] such as cell migration, morphology, and differentiation. For 3D cell culture within synthetic hydrogels, these properties are typically pre-engineered during gel fabrication or controlled post-fabrication through hydrolytic or enzymatic degradation of the network.[2] However, these systems lack user-defined, real-time control of material properties in space. Here, we offer a culture platform whose structure is tuned externally with light exposure.

Specifically, a photodegradable poly(ethylene glycol)-based (PEG) monomer was polymerized to form hydrogels that degrade upon light exposure, affording real-time spatial patterning in 3D. Photodegradable hydrogels were synthesized and subsequently degraded to demonstrate that gradients in structural properties and physical voids can be patterned into these gels. The degradation kinetics were characterized via photorheology and used to model the degradation. Adult hippocampal progenitor cells (AHPCs), whose process extension is dependent on scaffold structure,[3] were encapsulated within these gels and their response to structural gradients was examined by observing cell morphology.

Methods: A photodegradable monomer based on ethyl 4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic acid (photodegradable group, PD) was synthesized (PD-*b*-PEG-*b*-PD diacrylate, M_n~4070g/mol). Copolymer gels of PD-*b*-PEG-*b*-PD diacrylate and PEG monoactrylate (M_n~375 g/mol) were formed under redox-initiated polymerization (15 wt% monomer in water).

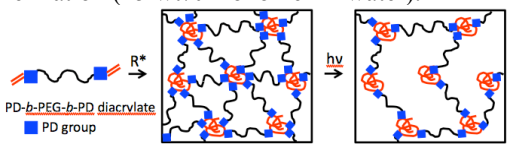

Figure 1: Photodegradable networks were formed by radical chain polymerization of PD-*b*-PEG-*b*-PD diacrylate and degraded by light exposure.

Thin films (50μm) were polymerized *in situ* between a quartz plate and a Peltier plate on a photorheometer and subsequently degraded while following the normalized shear modulus, which is equivalent to the normalized crosslinking density, to quantify the kinetics of the degradation. Channels of varying depths were patterned into the surface of these gels by exposing equilibrium swollen gels to 365nm light (12mW/cm^2) for varied lengths of time (5min to 30min). Confocal laser scanning microscopy (LSM) (Zeiss LSM 510, 405nm, ~25% intensity) was used to degrade 3D patterns within the middle of swollen gels. Structural gradients were formed through the depth of gels by exposing the surface to 365nm light (8mW/cm^2).

AHPCs were encapsulated within thin hydrogels (250μm) and subsequently exposed to 365nm light (8mW/cm^2) for 600s. AHPC morphology was evaluated at 1,6, and 10 days of culture at multiple locations within the 3D structural gradient.

Results: Photodegradable hydrogels were polymerized with an initial crosslinking density (ρ_x) of 0.017mol/L. When exposed to 365nm light (10mW/cm^2), ρ_x decreased as PEG was cleaved and released from the gel. This degradation was tracked with photorheometry, and ρ_x decreased to 0.0048mol/L after 250s of exposure, indicating that PD possesses a characteristic cleavage time (τ_{PD}) of 360s under exposure to 365nm light (10mW/cm^2). This implied that the kinetic constant of photolysis was 0.28 cm^2/Ws, which was used to predict the degradation with exposure to 365nm light.

400μm wide channels were patterned into these gels that increased in depth from 4μm at 5min of exposure to 75μm at 30min of exposure (Figure 2a.). 3D features, channels were successfully patterned within the gel using one-photon confocal LSM and subsequently visualized on the confocal LSM. Structural gradients within the gel were formed with ρ_x increasing from 0.0012mol/L at the surface of the gel to 0.015mol/L at a depth of 125μm in the gel after 600s of exposure to 365nm light (8mW/cm^2). (Figure 2b.)

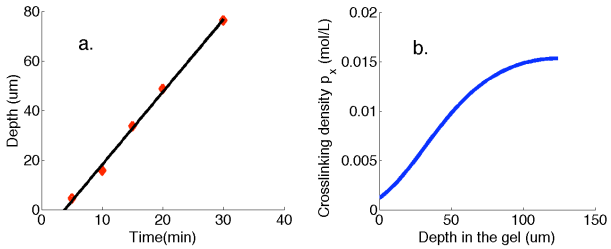

Figure 2: a. Depth of eroded channels as a function of exposure time. **b.** Crosslinking density as a function of depth within the gel.

AHPCs encapsulated within gradient gels showed an ability to extend processes only after the structure was relaxed with degradation. Furthermore, cells preferentially extended processes in the regions of gel with lower ρ_x.

Conclusions: Photodegradable hydrogels can be used as culture platforms whose 3D structural properties can be tuned in real-time. Physical voids and structural gradients can be patterned into these gels with photolithography and this platform shows promise for the 3D culture of AHPCs.

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References:

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