Polyethylene Glycol Diacrylate / Hyaluronic Acid semi-IPNs Support Increased Cell Spreading and Proliferation

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Statement of Purpose: Hydrogels based on polyethylene glycol (PEG) diacrylate modified with hydrolytically degradable bonds have been widely investigated as scaffolds for cartilage, bone, and vascular tissue engineering.[1] However, the 15 to 35Aº mesh size of these hydrogels[2] restricts cell spreading, resulting in a non-physiological, rounded morphology of encapsulated cells that can delay cellular growth, remodeling, and tissue formation. Several groups have addressed this limitation by using proteolytically sensitive peptide sequences as crosslinking agents.[3,4] The objective of these studies was to investigate an alternative approach for accelerating cellular remodeling based on the creation of semi-interpenetrating networks of PEG diacrylate and native, enzymatically degradable ECM components.

Methods: *Macromer synthesis* :- Degradable PEG ester acrylate macromers (PEGDA) were synthesized using the 2-chloropropionyl chloride monomer (Sigma-Aldrich, St. Louis, MO, USA) as described earlier[5]. *Cell spreading in semi-IPNs* :- 6% (w/v) solutions of the PEGDA macromer with varying types and amounts of ECM components, normal human dermal fibroblasts (4 x 10^6 / ml, NHDF, Biowhitaker), 2.5 mmol / ml acrylate-PEG-GRGDS, and 0.1% (w/v) I-2959 (Ciba) were crosslinked via UV exposure (6 minutes, 15 mW / cm^2).

Sample Name	Matrix Type	Matrix Concentration % (w/v)
PEGDA	None	0
PEG2GN	Gelatin	0.12
PEG2CO	Collagen	0.12
PEG1HA	Hyaluronic Acid	0.06
PEG2HA	Hyaluronic Acid	0.12
PEG3HA	Hyaluronic Acid	0.18

Hydrogel samples (n = 4 / group) were cultured in DMEM F-12 with 10% bovine growth serum. At 7 & 14 days, 2 samples per group were fixed in 4% paraformaldehyde, stained for actin using Alexa 594-phalloidin (Molecular Probes), and imaged at varying depths using a Zeiss Confocal LSM510 microscope. *Cell proliferation* :- NHDF (4 x 10^6 / ml) were encapsulated in PEGDA, PEG1HA, PEG2HA and PEG3HA semi-IPNs (N =6 per hydrogel type) and cultured as described above. N =3 samples from each group were harvested at 1 and 14 days and the cell number was determined using the Picogreen DNA-binding dye (Molecular Probes). *Swelling & Degradation studies* :- PEGDA, PEG1HA, PEG2HA and PEG3HA hydrogels were polymerized, equilibrated in water for 24 hours, freeze-dried, incubated in PBS at 37 °C, then freeze-dried and re-weighed at varying time points to measure swelling and mass loss. *Hyaluronidase activity*:- NHDF were encapsulated in 6%(w/v) PEG2HA and cultured 48 hours in the presence of the HAse inhibitors neomycin trisulfate hydrate (Aldrich, 0.1mM & 1mM) and ascorbic acid 6-palmitate (Aldrich, 0.1mM). HAse activity was measured by reverse HA gel zymography. Cell spreading in parallel cultures was visualized as described above.

Results/Discussion: Confocal microscopy images (Fig. 1) indicated that incorporation of 0.12 and 0.18%(w/v) HA facilitated cell spreading and migration within PEG-based semi-IPNs whereas addition of collagen, gelatin, and lower levels of HA (0.06%w/v) exhibited round morphologies consistent with PEGDA controls. When maintained in culture for 14 days, fibroblast number significantly increased in PEG2HA and PEG3HA gels, while a significant decrease was observed in PEGDA controls, suggesting some degree of cell death (Fig. 2). In order to investigate the mechanism by which HA incorporation increases cellular activity, we examined the hydrogel physical properties and HAse activity. PEG2HA and PEG3HA semi-IPNs exhibited significantly increased swelling (PEGDA Q=27.2 ± 1.6, PEG2HA Q= 37.3 ± 2.3) and a 10% increase in degradation rate (data not shown). Addition of both HAse inhibitors substantially reduced HAse activity and eliminated fibroblast spreading in PEG2HA semi-IPNs (data not shown).

Conclusions: These studies demonstrate that the addition of native HA to PEGDA hydrogels offers a simple method for increasing spreading and growth of encapsulated cells. HA incorporation affects both the physical and biological properties of the gels which may contribute to these observations. Through the use of HAse inhibitors, these studies demonstrate that cellular HAse activity is a necessary mechanism responsible for increased cellular activity in PEG / HA semi-IPNs. By accelerating early cellular remodeling and growth, these gels may be useful vehicles for cell transplantation in a variety of tissue engineering applications.

References: (1) Drury and Mooney. Biomaterials 2003; 24:4337-4351. (2) Cruise et al. Biomaterials 1998; 19: 1287-94 (3) West and Hubbel Macromolecules 1999; 32:241-244 (4) Lutolf and Hubbel Nature Biotech 2005; 23:47-55 (5)Datar et al. Society for Biomaterials (2005)

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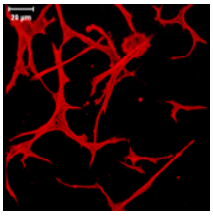

Figure 1 – Confocal image of encapsulated cells spreading within the 6%(w/v) PEG2HA (7 days)

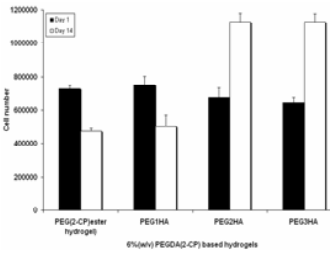

Figure 2 – Cell proliferation in 6%(w/v) PEG based semi-IPNs (14 days)