Hydrolytically Degradable Poly(Ethylene Glycol)-Laminin Hydrogel Scaffolds for Neural Progenitor Cell Delivery

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Statement of Purpose: Stem cell-based therapeutics for nerve injury and disease are currently challenged by poor control of graft survival and the guidance of specific cell responses including proliferation, migration, differentiation and integration into the injury environment. We present a promising strategy to pre-condition neural stem/progenitor cells (NPCs) within a hydrolytically degradable hydrogel scaffold based on cross-linked poly(ethylene glycol) (PEG). We demonstrated that gel properties are altered upon the physical incorporation of laminin within the gel structure; additionally, the presence of encapsulated NPCs further alters gel properties. The sum of these two effects was increased gel stiffness, decreased gel swelling and a slower degradation rate. The pre-conditioning environment of the laminin/gel scaffold was associated with increased cellular differentiation to the neuronal phenotype following release from the degraded gels. Thus, we report a system that offers the possibility of optimizing an array of gel bioactive and physical properties which may prove advantageous for future optimization of these scaffolds for specific clinical neurotransplantation applications.

Methods: Hydrogel synthesis and rheological and swelling measurements were adapted from our previous works.[1,2] Briefly, 4-arm PEG-vinyl sulfone (PEG-VS; 10 kDa) and laminin (Sigma-Aldrich) were mixed together to allow for final laminin concentrations of 0, 12 and 120 μM. This solution was then reacted with a hydrolytically degradable cross-linker, PEG- dithiopropionate (3.4 kDa) in a 1:1 ratio between the vinyl sulfone groups on PEG-VS and the thiols of the cross-linker. Gels were allowed to react at physiological conditions for 45-75 min (depending on gel size) to achieve maximum cross-linking. NPCs were derived from the cerebral dorsal telencephalon of E13.5 mice (Jackson Laboratory) and grown as neurospheres according to previously established methods.[3] For the cell delivery experiments (Fig.1), viable NPCs (p 2-6) were seeded in differentiation medium (serum-free DMEM/F12 media supplemented with B27 (Gibco)) onto laminin-coated coverslips (2D controls; ~2 μg/cm^2) at 20-25 neurospheres/cm^2 or embedded within 3D PEG-laminin gels at 7-10 neurospheres per 20 μl gel containing 0 or 12 μM (1% v/v) laminin.

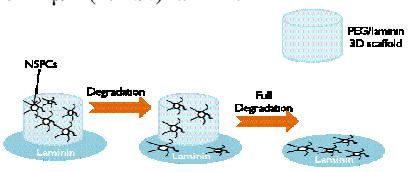

Fig. 1. Schematic of the cell culture system.

NPCs were cultured for up to 3 wks and then fixed in 4% formalin for immunocytochemistry analysis. The samples were incubated in primary antibodies against nestin (Invitrogen) or phenotypic markers for neurons (TUJ1) and astrocytes (GFAP) (Sigma-Aldrich) followed by appropriate fluorescently-conjugated secondary antibodies (Jackson Immunoresearch) and subsequent fluorescence imaging (Olympus IX81) and analysis.

Results: Gels containing 120 μM laminin had a lower G' (~10 Pa) than gels containing either 0 or 12 μM laminin (~16 Pa; Fig. 2); while this result was statistically significant we note that G' values are of the same order of magnitude. Incorporation of laminin did not significantly influence gel swelling (Qm; Fig 2). We also observed that gels containing laminin degraded in ~5d while unmodified PEG hydrogels degraded in ~3d. The presence of encapsulated cells significantly influenced the properties of all hydrogel types tested (Fig. 2). Hydrogel G' increased 2 to 5 fold, and swelling ratios decreased ~1.5 fold in all conditions. Because G' and Q_m are all indirectly related to the extent of cross-linking, these results suggest that the presence of encapsulated cells increases the effective cross-link density of the gels. As the gels degraded, cells were released from the hydrogels and adhered onto the laminin-coated coverslips placed below the gels. At 3 wks, cells expressing glial (GFAP+) and early neuronal (TUJ1+) markers were adhered to the laminin-coated coverslips for all conditions tested (Fig. 3). We can also observe that the 2D controls were associated with a significantly greater number of GFAP+ cells as compared to coverslips with cells released from gels containing 12 μM laminin, wherein the majority of cells were TUJ1+.

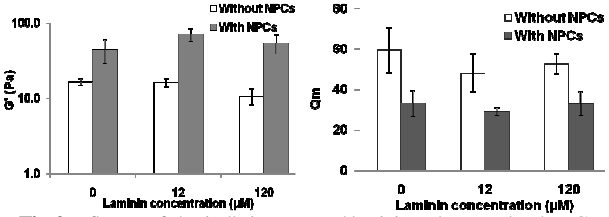

Fig. 2. Influence of physically incorporated laminin and encapsulated NPCs on the properties of PEG-laminin gels. NPCs were encapsulated in gels containing 0, 12 or 120 μM laminin and characterized for shear modulus (G') at 1 rad/s and swelling ratio (Qm). Bars represent average ± SD (n ≥ 4).

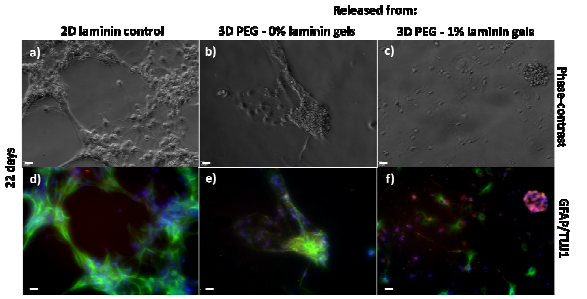

Fig 3 (a-c) Phase contrast and (d-e) fluorescent images show that NPCs were released from the gels, attached to the laminin surface at the bottom of the wells and after 22 d in culture differentiated into neurons (red) and glia (green). Cell nuclei were labeled with DAPI (blue). Bars, 10 μm.

Conclusions: These results suggest that pre-culture of NPCs within hydrolytically degradable PEG hydrogels with a continuous and controlled release of cells may be preferable to culturing NPCs directly on an adhesive substrate. The described hydrogel scaffold system offers the possibility of modifying an array of scaffold biological and physical properties, including the evaluation of soluble factors, adhesive ligands and degradation rates, which we and others have suggested to be potentially powerful means of modulating the efficacy of NPC-based therapeutics.[4,5]

References: 1. Zustiak SP and Leach JB, *Biomacromolecules* 2010, **11** 1348-57; **2.**Zustiak SP et al, *Acta Biomater*, 2010 **6** 3404-14; **3.** Bez A et al, *Brain Res,* 2003, **993** 18-29; **4.** Leach JL and Powell EM. Springer-Verlag Berlin Heidelberg; 2010; **5:** Lampe KJ et al, *Tissue Eng Part A,* 2010, **16** 1857-66.

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