The role of substrate stiffness on oligodendrocyte precursor cell growth *in vitro*

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Introduction: A major characteristic of multiple sclerosis (MS) lesions is the damage to the myelin sheath. Therapeutic strategies to enhance remyelination by increasing the number of oligodendrocytes capable of forming myelin in the lesion may be beneficial in treating MS. A growth-factor-dependent cell line of rat oligodendrocyte progenitor cell (OPC, CG4) is a valuable tool to study the development biology of myelin formation and the myelinating potential of OPCs. In order to maintain the growth factor (GF) level for OPCs at the transplantation site, a biomaterial suitable for long-term release of growth factor is needed. Previously, we found that long term release of GFs was possible using an injectable, in situ-crosslinkable ECM-based hydrogel based on thiol-modified hyaluronan (HA) and gelatin (Gtn) crosslined with poly(ethylene glycol) diacrylate (PEGDA), commercially available as Extracel (1). However, this series of hydrogels have never been used for OPCs. The stiffness of cell's environment impacts cell adhesion, proliferation, migration, differentiation and phenotype (2, 3). In order to select the optimal hydrogel for OPC transplantation, we tested the role of substrate stiffness on the CG4 cell viability, proliferation, and morphology in this study. By controlling crosslinking density, hydrogels with controlled stiffness can be obtained. CG4 cells were cultured both on the surface and encapsulated with hydrogels of different stiffness.

Material/Methods: CMHA-S (Glycosil) and Gtn-DTPH solutions (Gelin-S) (Glycosan BioSystems Inc. Salt Lake City, UT, USA) were prepared in sterile deionized distilled water under aseptic conditions. A 4.5% (w/v) PEGDA (MW3400, Extralink from Glycosan) stock solution was prepared by dissolving PEGDA powder in 1x PBS. Three volumes of CMHA-S solution, three volumes Gtn-DTPH solution and two volumes of cell culture media were mixed with two volume of PEGDA solution of varying concentrations (4.5%, 2.25%, 1.5%, 0.75% and 0%) to obtain hydrogels of different stiffness. An AR1000 rheometer (TA Instruments Inc. New Castle, DE, USA) with standard steel parallel-plate geometry of 40 mm diameter was used for the rheological characterization of all hydrogel samples. For cell cultured on hydrogel surface, hydrogel solution with different crosslink densities was added to cell culture wells. After 30 min 10,000 CG4 cell was added to each well. As to cell encapsulation into hydrogels, 10,000 cells mixed with hydrogel solution were added to cell culture well directly. The cells were cultured for 5 and 10 days in a 37^0C, 5% CO_2, 95% humidity incubator. LIVE/DEAD Viability/Cytotoxicity Kit (L-7013, Invitrogen) and Quantos Cell Proliferation Assay Kit (302011, Genetic Applications LLC. La Jolla, CA, USA) were used to investigate cell survival and growth depended on substrate stiffness. Imunocytochemistry is also used to characterize morphology and phenotypic plasticity of CG4 cells.

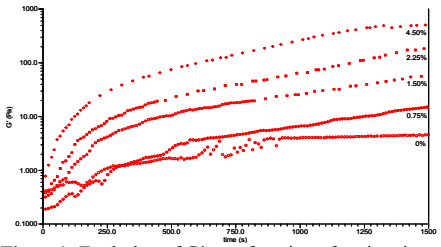

Figure1: Evolution of G' as a function of curing time.

Results/Discussion: Oscillatory time sweeps were performed to record the temporal evolution of shear storage moduli (G') of hydrogels. Figure 1 shows the time sweep profiles of G' for the 4.5%, 2.25%, 1.5%, 0.75% and 0% hydrogel networks within the small time frame. The development of G' appears to be solely governed by the PEGDA concentration. As shown in Figure 2 and 3, CG4 cells show similar survival on two-dimensional substrates and three-dimensional hydrogels of the same stiffness, but cell survival (Fig.2 and 3) and growth (Fig.3) decrease when substrate stiffness increases to a few hundred Pa. A dependence of cell shape on substrate stiffness is also observed from Figure 4 and 5. CG4 cells range from well spread to round as the stiffness is increased to a few hundred Pa. All of these data demonstrate that CG4 cells are inclined to grow on substrate with low stiffness (a few ten Pa).

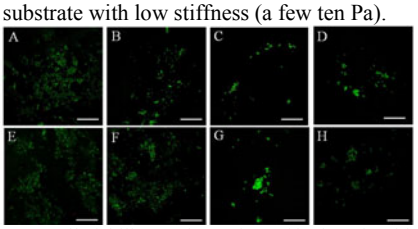

Figure 2: CG4 cell viability on the surface of hydrogels with different stiffness (A,E: 5 Pa; B,F: 28 Pa; C,G: 287 Pa; D,H: 383 Pa) at 5 (A-D) and 10 days (E-H). A-D: scar bar =300μm, E-H: scale bar=150 μm.

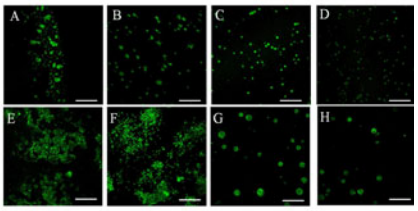

Figure 3: Viability of CG4 cells growing inside hydrogels of different stiffness (A,E:5Pa; B,F:28Pa; C,G:287Pa; D,H:383Pa) at 5 (A-D) & 10 days (E-H). A-D: scar bar=300μm, E-H: scale bar=150 μm.

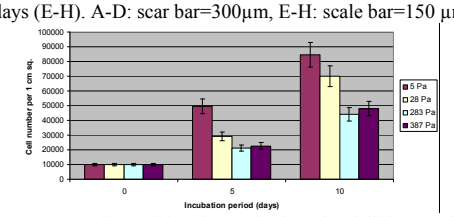

Figure 4: CG4 cells proliferation on hydrogels of different stiffness.

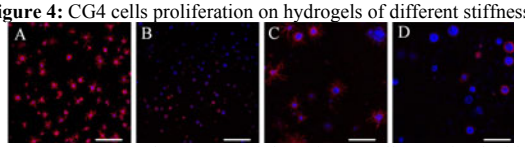

Figure 5: A2B5 and DRAQ5 staining of CG4 cells growing inside hydrogels of different stiffness (A, C: 28Pa; B, D:287 Pa) at 5 days (A, B) and 10 days (C, D). A, B: scar bar=300μm, C, D: scale bar=150μm.

Conclusions: Extracel, composed of CMHA-S, Gtn-DTPH and PEGDA, constitutes a valid system for making substrates with tunable mechanical properties. CG4 cells are inclined to grow on this substrate with stiffness of a few tens of Pa.

References:

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