Engineered Hyaluronic acid based Hydrogels for Survival and Transplantation of Stem Cells

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Introduction: Recently various cell-based therapies have been developed for the treatment of damaged or diseased tissues; however low cell survival and poor engraftment have resulted in limited success of these therapies. To improve the cell survival and engraftment, we have developed a novel hydrogel system of hyaluronic acid (HyA) that contain peptide sequences for cell attachment via binding of integrin receptors, heparin for presentation and modulation the sequester characteristics of exogeneously added growth factors and retention of endogeneously produced growth factors, and enzymatically degradable matrix metalloproteinase (MMP) sensitive peptide crosslinks.[1, 2] In this work, we have investigated the role of hydrogel components to promote cell survival, adhesion, endothelial cell differentiation and tubule formation using endogenous Sca-1$^+$/CD45$^-$ cardiac progenitor cells (CPCs). Optimized HyA hydrogels were used to implant CPCs subcutaneously in murine hindslimb to evaluate the survival, and engraftment of CPCs.

Methods: An HyA (Mw 500kDa) derivative carrying hydrazide groups (HyAADH) was synthesized using previous methods,[3] and acryloxysuccinimide (700 mg) was subsequently reacted to the HAADH solution (300mg, 100 mL DI water) to generate acrylate groups on the HyA (AcHyA). The AcHyA-RGD derivative was synthesized by reacting CGGNGEPRGDTYRAY (bsp-RGD (15)) (10mg) with a AcHyA solution (25mg, 10mL DI water) at room temperature. Separately, thiolated-heparin was synthesized by reacting heparin (50mg, 10mL DI water) with an excess of cysteamine in the presence of EDC and HOBt at pH 6.8. Hydrogels were made by mixing AcHyA (4mg), AcHyA-RGD (6mg), and heparin-SH (0.03 wt %) dissolved in 0.3 mL of triethanolamine-buffer (TEOA; 0.3 M, pH 8), and MMP-13 (CQPQGLAKC) cleavable cross-linker. Viscoelastic properties of the hydrogel were determined by an oscillatory rheometer under 10% constant strain and frequency ranging from 0.1 Hz to 10 Hz. CPCs were encapsulated in the hydrogel at 5 million cells/mL. The cell viability in hydrogel was assessed by a live/dead assay, cell attachment was characterized by F-actin/vinculin staining, and cell phenotype was characterized by anti-CD31 immunostaining. Then, encapsulated CPCs in HyA hydrogels were implanted in the subcutaneous region of murine hinds limb to assess *in vivo* cell survival and engraftment with host tissue .

Results: Synthesized AcHyA had ~ 28% conjugation of acrylate groups on the repeating units of HyA chains. The acrylate groups on AcHyA were used as the reactive handles for bioconjugation and crosslinking. AcHyA-RGD was prepared by a Michael Type I addition reaction between the cysteine of bsp-RGD(15) and acrylate groups of available AcHyA. Thiolated-heparin was synthesized by conjugating cysteamine to the carboxylic groups of HyA using carbodiimide chemistry. Subsequently, these precursors of HyA were crosslinked in situ using an enzymatically degradable MMP-13 sensitive peptide containing cysteine at the both ends of the peptide.[4] Viscoelastic storage moduli of these hydrogels were tuned from 10Pa to 850Pa. Covalent conjugation of heparin (0.03 wt%) in the HyA network retained upto 70% of the TGFβ1 for three weeks. Subsequentely, HyA hydrogels were used to investigate the influence of matrix paramters on survival, proliferation and vascular tube formation via the differentiation of endogenous cardiac progenitor Sca-1$^+$CD45$^-$ cells (CPCs) into the endothelial cell lineage. *In vitro* encapsulated Sca-1$^+$CD45$^-$ CPCs within the hydrogel network were viable, proliferated and formed vessel-like networks. Excess of immobilized heparin within HyA hydrogel was able to retain endogeneously produced angiogenesis related proteins by CPCs. And, cell proliferation and tube formation can be tuned by altering the peptide density and modulus of the hydrogel.. *In vivo*, HyA hydrogels promoted CPC survival and neovascularization when implanted in the subcutaneous region of murine hind limbs. Therefore, we anticipate these HyA hydrogels are promising candidates for the application of cell transplantation.

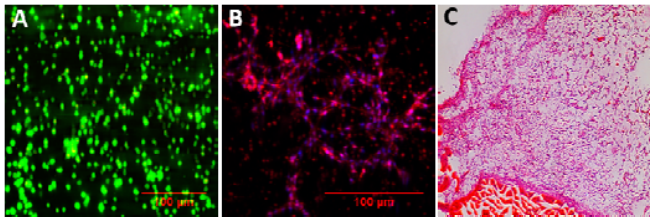

Fig. 1. Representative A). confocal microscopy image of live/dead staining of CPCs in the sIPN (live cells: green and dead cells: red), B). confocal microscopy image of CD 31 immunofluorescence of CPCs in the sIPN (CD31: red, cell nuclei: blue) and C).bright field image of H&E stain of explanted hydrogel from murine hinds limb after 12 days.

References:

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