Controlling the Adhesion and Differentiation of Mesenchymal Stem Cells Using Cell Responsive, Hyaluronic Acid-based, Doubly-Crosslinked Networks

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Statement of Purpose: Biomimetic scaffolds that recapture the key features of the native extracellular matrices and foster cell-matrix interactions are indispensible for the successful engineering of function tissues *in vitro*. We have developed hyaluronic acid (HA)-based doubly crosslinked networks (DXNs) consisting of densely crosslinked HA hydrogel particles (HGPs) embedded in a loosely connected secondary matrix that is also HA-based.[1-2] Such hierarchically-structured HA matrix allows for the controlled release of morphogenic factors. Primary chondrocytes encapsulated in this matrix not only maintained their chondrogenic phenotype but also produced cartilage-specific ECM components.[3] However, the hydrophilic nature of HA limits the utility of the DXNs for the culture of anchorage-dependent cells. Thus, cell-adhesive HA DXNs were fabricated by encapsulating gelatin-decorated HA HGPs in a secondary HA matrix. Human mesenchymal stem cells (MSCs) were shown to adhere readily to the composite matrix through the focal adhesion sites clustered on particle surface. The cell-adhesive composite matrices support the proliferation, migration and osteogenic differentiation of MSCs.

Methods: HA-based HGPs were synthesized following a previously reported procedure.[1-2] Gelatin was conjugated to HA HGPs in PBS at pH 7.4 in the presence of $NaBH_3CN$. The gelatin conjugated HGPs (G-HGP) were allowed to react with glycine in water for 2 h at 37 °C under constant mixing. Separately, photocrosslinkable HA was synthesized by chemical modification of HA with glycidal methacrylate (HA-GMA).[3] Dry G-HGPs were dispersed in HA-GMA (2wt% in DI H_2O) at a concentration of 25 mg/ml. To this solution was added 1.5 µL of the initiator solution [30% 2,2-dimethoxy-2-phenylacetophenone (DMPA) in 1-vinyl-2-pyrrolidinone (NVP)]. The mixture was exposed to a long wavelength UV lamp for 15 min for complete gelation. HA-GMA gels without any particles were included as the controls. MSCs were plated on various gel disks and were cultured in MSC growth media. The matrix toxicity was assessed by live/dead assay, cell proliferation was analyzed by alamar blue assay and cell attachment was characterized by F-actin/vinculin staining. Osteogenic differentiation was assessed by Alizarin Red S, VonKossa and Collagen I staining.

Results/Discussions: The as-synthesized HA HGPs have an average diameter of 10 µm and contain readily accessible functional groups for bioconjugation purposes.[4] Thus the aldehyde groups on/in HGPs were utilized as the reactive handles for the immobilization of gelatin through the lysine amines via a reduction amination reaction. After gelatin conjugation, the unreacted aldehyde groups of HGPs were passivated using glycine. Hydroxyproline assay shows that G-HGPs contain an average 22.45 ± 1.36 µg gelatin per milligram particles. Gelatin conjugation was further confirmed by fluorescamine staining. HGPs without gelatin conjugation were used as the negative controls. The excess hydrazide groups[1] in HGPs and G-HGPs were deactivated by acetaldehyde prior to the assay so as to eliminate the complication introduced by hydrazide groups. The positive flourescamine staining of G-HGP confirmed the successful gelatin conjugation while the negative staining from the control samples indicates the complete blocking of the active hydrazide groups.

Gelatin-conjugated HA HGPs were embedded in HA-GMA matrix by UV-induced radical crosslinking. The resulting hydrogel contains microscopic particles distributed in a nanoporous matrix. Compared to the secondary matrix, the embedded HGPs are significantly more crosslinked. Live/dead assay shows (data not shown) that the HA matrices are non-toxic to the cells. Furthermore, the cell number increased by a factor of 2.5 over 2 weeks of culture. MSCs cultured on G-HGP containing matrix readily attached to the gel through the focal adhesion sites on the particles. After 1, 3 days of culture, cells exhibit distinct spread-out morphology with extended actin fibers and well-defined focal adhesion. By day 14, cells have already populated the surface and established a coherent community. Contrarily, cells plated on the control gels (HA-GMA) form isolated spheroids without the defined stress fiber (Figure 1). Immunostaining confirmed that MSCs cultured on HA-G-HGP were differentiated into osteoblasts after 28 days of culture in the MSC growth medium.

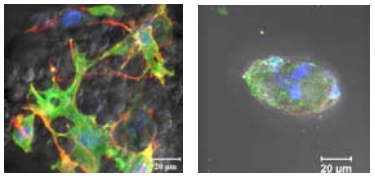

Figure 1. Representative focal adhesion and F-actin staining of MSCs after 14 days of culture on HA-G-HGP (left) and HA-GMA (right). F-actin filaments: red; vinculin: green; cell nuclei: blue.

Conclusions: HA DXNs containing gelatin-conjugated HGPs were successfully synthesized by photopolymerization of soluble HAGMA in the presence of G-HGPs. The resultant DXNs support the attachment, proliferation and osteogenic differentiation of human mesenchymal stem cells.

References:

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4. Jha, A. K. et al. *Biomaterials,* **2009**, *30*, 6964.