Hyaluronic Acid-Based Hydrogel Particles and Doubly Crosslinked Networks for Soft Tissue Regeneration

Amit K. Jha[1], Weidong Yang[2], Catherine B. Kirn-Safran[2], Mary C. Farach-Carson[1,3] and Xinqiao Jia[1]
[1]Department of Materials Science and Engineering, [2]Department of Biological Sciences, University of Delaware
[3]Departmemt of Biochemistry and Cell Biology, Rice University

Statement of Purpose: Hyaluronic acid (HA) is the only non-sulfated glycosaminoglycan (GAG) in the natural extracellular matrix (ECM), playing vital roles in wound healing, cell differentiation, and cell motility. Due to its biocompatible and biodegradable nature, HA has been widely used for biomedical applications.[1] We are interested in engineering HA-based matrices with hierarchical structure, tunable viscoelasticity, improved enzymatic stability and spatio/temporal presentation of growth factors for soft tissue regeneration. To this end, HA-based hydrogel particles (HGPs) with defined size and porosity were prepared via inverse emulsion polymerization.[2-4] When conjugated with perlecan domain I (PlnDI), these particles allow for controlled release of therapeutically active growth factors.[5] Hierarchically structured, doubly cross-linked networks (DXNs) were engineered using HA HGPs as the building blocks and a water soluble HA derivative as the secondary cross-linker.[2-4] The resulting hydrogels are soft and elastic, exhibiting unique frequency-dependent viscoelasticity. These artificial ECM are attractive candidates for biomedical applications.

Methods: The synthesis and characterization of HA derivatives carrying aldehyde (HALD) and hydrazide (HAADH) groups, HA HGPs and DXNs can be found in our previous publications.[2-4] PlnDI was conjugated to HA HGPs through a flexible poly(ethylene glycol) (MW 3400) linker using reductive amination under slightly acidic conditions. BMP-2 loading and release from PlnDI-conjugated HGPs were monitored using BMP-2 ELISA kit.[5] HA DXN was obtained by direct mixing of HA HGPs with HAADH. The viscoelastic properties of HA DXNs were evaluated using a custom-built torsional wave apparatus.[2]

Results/Discussion: HA HGPs with an average diameter of 10 μm were synthesized via an inverse emulsion polymerization process using HA derivatives carrying complementary functional groups (HAADH and HAALD). In vitro cytotoxicity studies using vocal fold fibroblasts indicate that the HA HGPs are essentially non-toxic.[2] The particles are enzymatically more stable than their corresponding macroscopic counterparts. PlnDI, capable of sequestering BMP-2 and modulating its release in natural ECM, was covalently conjugated to HA HGPs (Figure 1) in order to control the release kinetics of BMP-2. Prior to PlnDI conjugation, a flexible PEG linker was introduced to HGPs, converting the hydrazide groups on HGPs to aldehyde. PlnDI was conjugated to HGPs via reductive amination using the free amines present in the core protein of PlnDI and the terminal aldehyde groups on PEG that has been anchored to HGPs. The immobilized PlnDI maintained its ability to bind BMP-2 specifically. Furthermore, PlnDI-conjugated HGPs exhibit a significantly higher BMP-2 binding efficiency (168 ng/mg) as compared to HGP alone (89 ng/mg). The in vitro release results showed that approximately 65% of BMP-2 was released from HGP in three days. Contrarily, a sustained release was observed for BMP-2 loaded, PlnDI-conjugated HGPs. Only 18% of BMP-2 was released in the first three days, thereafter an average of 2% release was achieved per day until day 15 when the experiment was terminated. Finally, the released BMP-2 caused the chondrogenic differentiation of mesenchymal stem cells in micromass culture format (data not shown).

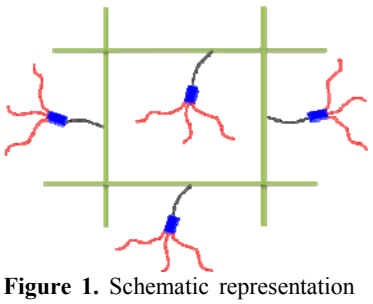

Figure 1. Schematic representation of PlnDI-conjugated HA HGP. Green lines: Crosslinked HA network in HGPs; black lines: PEG linker; blue squares: PlnDI core protein; read lines: heparan sulfate side chains. Not drawn to scale.

The presence of residual functional groups allows for subsequent crosslinking of HGPs with HAADH, giving rise to doubly crosslinked networks (DXN) with tunable viscoelasticity. In our crosslinked microgel networks, the individual particles are highly crosslinked, making them resistant to degradation, while macro-scale mechanical properties can be independently tunable by adjusting HGP dimensions or inter-particle crosslinking. Moreover, the HGPs have a relatively large surface area, which might improve tissue integration and facilitate controlled delivery of therapeutics. Finally, the presence of two levels of crosslinking (within and between individual HGPs) may offer potential for rapid recovery from mechanical stress. These HA-based HGP and DXN systems are promising candidates for the use as injectable materials in wound healing, adhesion prevention and soft tissue engineering.

Conclusions: we have created a new class of biocompatible materials based on HA HGPs that exhibit controlled particles sizes, improved enzymatic stability, defined biological functionalities and tunable mechanical properties. These microgel systems are promising candidates as injectable materials for soft tissue regeneration.

References:

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