Self-assembled and Nanostructured Hydrogel Scaffolds as New Bone Substitutes

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Statement of Purpose: The objective of the current in vitro study was to create an injectable and nanostructured 3D scaffold based on the novel self-assembled properties of helical rosette nanotubes (HRNs) to fill bone fractures and repair bone. The new bone substitutes possess not only suitable mechanical properties (through the incorporation of hydrogels) but also cytocompatible properties (through the incorporation of HRN). Although traditional methods (such as autografts and allografts) to treat bone defects have been performed clinically, there still exists many shortcomings such as donor site morbidity, donor tissue shortage for autografts and inflammation and transmission of diseases for allografts. Thus, scientists are pursuing the development of a new generation of biocompatible bone tissue-engineering alternatives: for instance, the formation of injectable nanostructured scaffolds onto the site of fracture is a promising and easy-to-use method. HRNs are novel soft organic nanotubes 3.4 nm in diameter obtained through the self-assembly process of DNA base pair building blocks in water. Because HRNs can undergo a phase transition from a liquid to a viscous gel when heated or when added directly to serum-free media at body temperatures, HRNs may provide an exciting therapy to heal bone fractures and defects as an injectable scaffold. Furthermore, HRNs have better cytocompatibility and can remarkably improve osteoblast (bone-forming cell) adhesion when coated on traditional implant materials [1-2]. In order to achieve more robust scaffolds, we embedded and coated HRNs in and on hydrogel matrices (pHEMA) which have already been extensively used in conventional forms as tissue engineering and drug delivery materials [3].

Methods: In the study, HRN-K1 (with lysine side chain) embedded hydrogel scaffolds were prepared by mixing HRN-K1 in solution with the same volume of 2-hydroxyethyl methacrylate (HEMA) monomer as well as 2, 2'-azobisisobutyronitrile initiator in a 10 ml vial [4]. Then, the mixture was heated in an oven at 45°C for less than two hours to polymerize. HRN coated hydrogel scaffolds were also prepared by simply adsorbing HRN-K1 on hydrogels. The gelling conditions of various scaffolds were studied. Moreover, HRN-K1 hydrogel scaffolds were characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy(AFM). Furthermore, human fetal osteoblast adhesion was tested on five types of HRN-K1 hydrogel scaffolds. Osteoblasts (PN#9; ATCC) were seeded at 3500 cells/cm^2 and cultured in DMEM/F-12 Ham supplemented with 10% fetal bovine serum, 1% Penicillin-Streptomycin under standard cell culture conditions (37°C, humidified, 5% CO_2/95% air) for 4 h.

Results and Discussion: Enhanced osteoblast adhesion on HRN-K1 embedded and coated hydrogel scaffolds, even at a very low HRN-K1 concentration of 0.001 mg/ml, was observed in the present study (Figure 1). Hydrogel scaffolds coated with 0.01 mg/ml HRN-K1 increased osteoblast adhesion 129.5% compared to uncoated hydrogel controls. These results are consistent with previous studies that focused on the investigation of HRN-K1 as a potential orthopedic coating material [1-2]. Higher concentrations of HRN-K1 hydrogel scaffolds may provide more lysine side chains on the biomaterial surface, thus, possibly mimicking lysine-rich bovine bone proteins known to enhance osteoblast functions [5]. Also, it was found that HRN-hydrogel scaffolds need significantly less polymerization time than hydrogel controls, especially at low temperatures (40-45°C). That is, by incorporating HRN-K1 with hydrogels, our results showed that bone regenerative constructions can be made through their solidification into viscous materials in less than 120 minutes at 40 °C (220 minutes for only hydrogels). In summary, it is evident from these results that the HRN-K1 hydrogel scaffolds (both coated and embedded) have the potential to be used as injectable scaffolds for tissue engineering applications and, thus, warrant further attention.

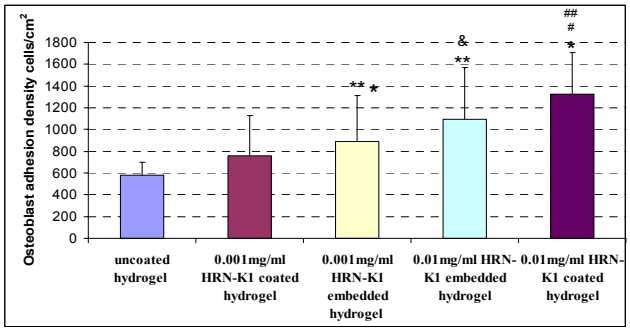

Figure 1. Improved osteoblast adhesion on HRN-K1 coated and embedded hydrogels. Data are mean ±SEM; n=3. * $p<0.001$, ** $p<0.01$, *** $p<0.05$ when compared to uncoated hydrogels; # $p<0.005$ when compared to 0.001 mg/ml HRN-K1 coated hydrogels; ## $p<0.05$ when compared to 0.001 mg/ml HRN-K1 embedded hydrogels; and & $p<0.1$ when compared to 0.001 mg/ml HRN-K1 coated hydrogels.

Conclusions: HRN-K1 hydrogel scaffolds (both coated and embedded), especially at 0.01 mg/ml HRN-K1 concentrations, promote osteoblast adhesion due to their nanoscale dimensions and chemistry. Easy and fast preparation of HRN-K1 embedded hydrogels makes them clinically promising materials.

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