Fluorenylmethyloxycarbonyl-based Peptide-Chitosan hybrid Scaffolds for Potential Applications in Cartilage Tissue Regeneration

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Statement of Purpose: Regenerative medicine is a promising field which utilizes the transplantation of xenogeneic tissues and organs to patients in need. However, challenges including infection, graft rejection, immunosuppression and adverse effects of antimicrobial drugs coupled with a limited organ supply deter the success rate of these procedures.[1,2] To overcome these challenges, tissue engineering (TE) has emerged as a promising method for reconstructive medicine centered around the manipulation of cells, scaffolds and stimuli to regenerate tissues that could restore functions lost due to disease, trauma or aging.[3] Progress has been made in the fabrication of host-tissue based bone, skin, bladder and blood vessel implants.[4] However, work in the field of cartilage TE has remained elusive due to its avascular nature and inability to elicit a healing response. Thus we have developed a scaffold-based approach for preparation of biomaterials with potential applications in cartilage tissue regeneration. Specifically, self-assembled nanoscale fluorenylmethyloxycarbonyl (Fmoc) protected valine-based hydrogels were layered with peptide sequences with high affinity for chondrocytes, and polysaccharides such as chitosan as well as glycosaminoglycans such as chondroitin sulfate to mimic the extracellular matrix of cartilage tissue. Our results indicate that the formed scaffolds were found to be biocompatible and successfully adhered to mammalian chondrocyte cells as well as supported cell proliferation. Such nanoscaffolds may have potential applications in cartilage tissue regeneration.

Methods: Fmoc-Val-cetylamide hydrogels were prepared according to previously established methods.[5] The self-assembled hydrogels (500 µl) were incubated with 200 µl of dentin sialophosphoprotein binding peptide sequence and stirred for 30 minutes. The mixture was allowed to stand for an hour at 4°C followed by the addition of collagen (200 µl). The mixture was then shaken for an hour at 4°C. The solution was incubated overnight and centrifuged for an hour to remove any unbound peptide. Next, chitosan (200 µl, 0.1 M) from rooster comb, purchased from Sigma Aldrich, was added and allowed to incubate overnight. Finally, chondroitin sulfate in water (300 µl, 0.05M) was added and the solution was shaken and incubated for 24 hours before further characterization. The morphologies of the assemblies were characterized using TEM (JEOL 120 X) and SEM (Zeiss-EVO 10) . Confirmation of incorporation of the peptides as well as chitosan and chondroitin sulfate was also carried out using FTIR spectroscopy (Thermo Scientific, Nicolet IS50). To examine the effect of the nanoscaffolds on chondrocyte growth, cell proliferation assays were conducted over a period of 96 hours. Briefly, bovine chondrocyte cells (Astarate Biologics) were plated in culture medium in 24-well Costar plates at a density of 6 $\times 10^4$ cells per well. After allowing the cells to attach and spread for 2 hours, nanoscaffolds of varying quantities (20 µl to 100 µl) were added into wells containing 1mL final volume of DMEM media and incubated with the cells for an additional 48 to 96 h. To determine cell viability, the adherent and any unattached cells were collected from each well by trypsinization and the number of live cells was determined by trypan blue exclusion.

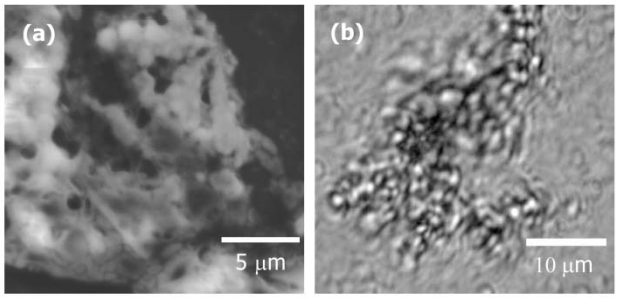

Figure 1. (a) SEM image of nanoscaffold; (b) Optical microscopy image showing attachment of nanoscaffolds to chondrocytes after 96 hours of incubation.

Results: Fmoc-Val-cetylamide hydrogels demonstrated nanofibrillar structures upon self-assembly, owing to the aromatic stacking interactions of the Fmoc moiety. The hydrophobic nature of valine and the long, hydrocarbon tail of cetyl amide also allowed for hydrophobic interactions resulting in assembly. We found nanofibers in the range of 200-400 nm in diameter and after the addition of each respective layer, three-dimensional nanoscaffolds were formed as shown in Figure 1a. FTIR analysis showed the subsequent addition of each layer with significant shifts in the amide I region owing to the incorporation of each layer to the scaffold. Cell studies in the presence of chondrocyte cells showed attachment to scaffolds (Figure 1b) under optical microscopy while trypan blue studies indicated that the cells continued to proliferate over time. Separate chondrogenesis assays were also conducted that confirmed proteoglycan synthesis.

Conclusions: A new nanoscale biomimetic peptide-based hydrogel nanoscaffold was prepared by the layer-by-layer assembly method. The interactions of the scaffolds with chondrocyte cells was studied. The nanoscaffolds exhibited biocompatibility, induced proliferation of chondrocyte cells and promoted chondrogenesis. Such nanoscaffolds may have potential applications in cartilage tissue regeneration therapeutics.

References:

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