Protein Based Biocomposite Cryogels as Tissue Engineering Scaffolds

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Statement of Purpose: In bone tissue engineering applications, natural bone structure is followed as a guide for generate composite materials with good mechanical and biocompatibility properties. Therefore, recent studies focus on three-dimensional scaffold materials which show good biomimicry of the bone tissue and provide suitable environment for bone tissue formation. [1] In this study, cryogelation method was chosen to produce gelatin and ovalbumin scaffolds. Because, cryogelation method provides the production of scaffolds with highly and controllable porous structure. Moreover, novel ovalbumin/hydroxyapatite scaffolds were produced by this method.

Methods: At first, by using glutaraldehyde as a crosslinker, gelatin cryogels were prepared. Two series of cryogels were synthesized by varying the gelatin concentration and glutaraldehyde amount. Also, another series of cryogels were prepared by incorporating different amounts of hydroxyapatite within cryogels to obtain biocomposites which can be good candidate biomaterials for hard tissue repair. Then, ovalbumin cryogels were also investigated with the same parameters except it was dissolved in urea solutions. All produced cryogels were characterized by gel fraction, equilibrium swelling, swelling kinetics, mechanical properties and internal morphology measurements. The porosity of the cryogels was observed by scanning electron and light microscopy. Finally, produced cryogels were combined with cells *in vitro*. Biocompatibility and osteoinductivity of cryogel scaffolds were investigated using 10T1/2 fibroblast cells and MG63 osteoblast cells by Presto Blue and Live/Dead staining.

Results: In the terms of mechanical compression experiments, ovalbumin cryogels showed good mechanical properties but gelatin cryogels were so weak. This was also mentioned before in somewhere else. [2] Swelling ratios calculations and scanning electron microscope experiments indicated that we had gels with highly macroporous structure in both types. It was noted that polymer, crosslinker and hydroxyapatite concentrations are important parameters for synthesis of good cryogel scaffolds using gelatin and ovalbumin. Moreover, cell culture experiments did not gave good results. After following days, cells started to die.

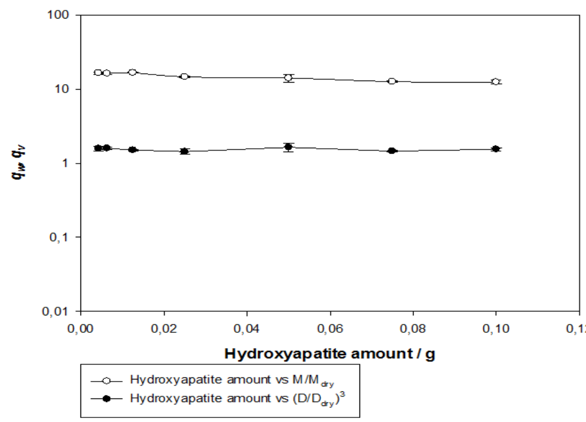

Figure 1. The equilibrium weight and volume swelling ratios for ovalbumin cryogels respect to dry state shown as a function of hydroxyapatite amount.

Conclusions: Ovalbumin cryogels showed better mechanical properties and more stable pore structure than gelatin cryogels and the stability was increased by increased polymer and crosslinker concentrations. All produced cryogels exhibited superfast swelling behavior. Addition of hydroxyapatite increased the mechanical properties of gelatin gels which were more stable and cylindrical but for ovalbumin gels it was not so effective. For ovalbumin gels, pore diameter was increased by increased polymer concentration. Moreover, cell culture studies did not give satisfactory results in all sets of gels. But glutaraldehyde crosslinked gels showed better cell proliferation. We discussed that the reason of the cell lost could be due to insufficient washing conditions. The future studies will be focused on to solve this problem.

References: 1. Song, V. Malathong, C.R. Bertozzi, 2005, J Am Chem Soc 127 3366-3372.**2.** Mattiasson, B., and et al., 2012, J Mater Sci: Mater Med, 23, 2489–249.