Characteristics of Heparin-functionalized Porous PLGA Scaffold for Tissue Regeneration

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Statement of Purpose: The aim of this study is to develop highly functional biomimetic scaffolds with specific bioactivity by using novel porogen and plasma treatment for cartilage tissue engineering. Solid hydrogen peroxide compounds, such as urea hydrogen peroxide and sodium percarbonate are well-known commercial products used as a bleaching agent, antiseptic and disinfectant. Urea hydrogen peroxide is an odorless, nontoxic and white crystalline powder which releases hydrogen peroxide (H_2O_2) locally on application. It is highly soluble in water and has both the character of H_2O_2 and urea. The hypothesis is the use of solid H_2O_2 as a novel porogen would be beneficial in making 3D porous and functional scaffolds for tissue regeneration. The additional application of heparin, a polysaccharide macromolecule associated with the cell surface and extracellular matrix, can specifically bind with numerous biologically proteins such as growth factors, thereby playing an essential role in the regulation of various biological signaling. Therefore, heparin-grafted scaffolds can create a more biomimetic microenvironment in the body for functional tissue regeneration. In this study, porous PLGA scaffolds were fabricated using a gas forming technique with urea hydrogen peroxide as a novel porogen. The surface of the scaffold was then grafted with heparin. The physical and biological properties of the protein modified scaffold were investigated.

Materials and Methods: Porous PLGA scaffolds were fabricated by a gas forming technique using a urea hydrogen peroxide with different contents. The surface of fabricated porous scaffolds was modified using argon plasma treatment and *in situ* acrylic acid (AA) graft polymerization. The resultant carboxyl groups were readily reacted in a mild aqueous solution, containing EDC/NHS/MES buffer (pH 5.6), followed by the grafting of heparin. The properties of the heparin-grafted scaffolds were analyzed by Mercury porosimetry, ATR-FTIR, SEM, Toluidine blue O measurement. Chondrocytes were then seeded onto either Heparin-grafted PLGA scaffold or control PLGA scaffold and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin. The cell-substrate interactions were examined using various methods, including SEM observation and WST viability assay.

Results: The surface morphology of the porous scaffolds was observed by SEM. The fabricated scaffolds demonstrated an open and interconnected pore structure with no structural delaminations on the external surface as shown Figure 1.

When porosity and pore diameter were measured by mercury porosimetry, the maximum porosity was 80% for PLGA scaffold with a porogen/polymer weight ratio of 20:1 (c) and a corresponding average pore size between 50 and 300 μm. Using a ratio of 10:1 (a), the scaffold had lower porosity (70%) and reduced numbers of pores on its surface. A ratio of 15:1 (b) was an optimal condition to produce porous PLGA scaffold using urea hydrogen peroxide. An analysis of the heparin-grafted scaffold by ATR-FTIR, showed a specific peak corresponding to the hydroxyl group in the heparin molecular. From the WST viability assay, the heparin-grafted PLGA scaffold demonstrated stronger ability to induce chondrocyte proliferation than the control PLGA scaffold. These results show that heparin-grafted porous PLGA scaffolds can enhance the cell-biomaterial interaction.

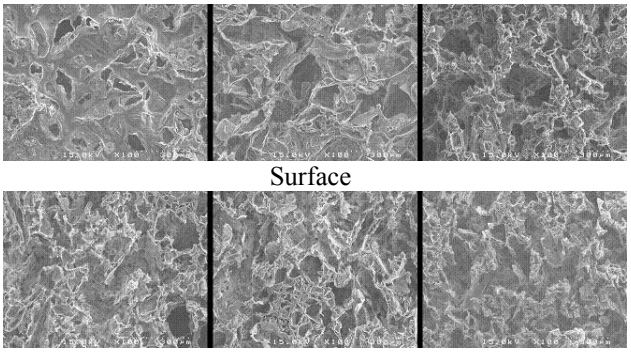

Figure 1. SEM images of porous PLGA scaffolds (100 x): (a) porogen/polymer weight ratio 10:1, (b) 15:1, (c) 20:1.

Conclusions: Heparin-grafted porous PLGA scaffold was successfully prepared by a gas forming technique with urea hydrogen peroxide. Utilizing varying processing parameters, fabricated scaffolds showed a porosity of 70-80%, pore size of 50-300 μm. Proliferation of chondrocytes in the heparin-grafted PLGA scaffold was improved compared to the control PLGA scaffold. We conclude that, heparin-grafted PLGA scaffolds are more bioactive and suitable for chondrocyte culture, which is a potentially applicable material to tissue regeneration for condrogenesis and cartilage wound repair.

References:

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