Zonal Release of Proteins within Tissue Engineering Scaffolds

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Abstract

A novel scaffold that achieves zonal growth factor release was developed to stimulate the regeneration of two or more tissue types. This scaffold was assembled from microparticles to control the release of tissue-specific growth factors, into distinct zones within a 3D multi-layered structure, and to direct the actions of endogenous or transplanted cells into different functional tissues. Poly(D,L-lactic acid) ($P_{DL}LA$) microparticles were loaded with model proteins, trypsin and horseradish peroxide, or recombinant human bone morphogenetic protein - 2 (rhBMP-2). Encapsulation efficiencies and retention of activities higher than 75% were achieved using a solid-in-oil-in-water method.

Microparticles were fused into 3D scaffolds using heat sintering and pressurized carbon dioxide (CO_2). In the first method, the incorporation of a plasticizer poly(ethylene glycol) was shown to improve the process and the mechanical strength of the scaffolds. In the second method, it was shown that the use of pressurized CO_2 allowed microparticle fusion at room temperature and low pressure. Strong scaffolds with highly interconnected open pores and preserved particle microstructures were obtained by manipulating the pressure, pressurization period and temperature. Encouragingly, the pressurized CO_2 process was developed without salt leaching manipulation or addition of a stabiliser. Controlled release of active proteins from both scaffold types was achieved for four weeks. Protein release kinetics from microparticles was maintained following the pressurized CO_2 fusion, however, it was retarded after the heat sintering. In addition, alkali treatment of microparticle based-scaffolds was shown to enhance cell adhesion, which could improve scaffold functionality.

Zonal release scaffolds were then fabricated by the assembly of protein loaded and protein-free microparticles into different layers within 3D structures, followed by fusion using both heat sintering and pressurized CO_2. It was shown that the release of proteins was restricted to zones within these scaffolds. Zonal activity of rhBMP-2 on

C2C12 cells was demonstrated on both scaffold types. Cell response to rhBMP-2 was tuneable by changing the dose of the released rhBMP-2, which was achieved by varying the ratio of protein-loaded and protein-free microparticles within the scaffolds. Zonal activity of rhBMP-2 was higher on pressurized CO_2 scaffolds than on heat sintered scaffolds containing the same ratio of rhBMP-2 microparticles. Therefore, pressurized CO_2 is the selected method for the fabrication of zonal release scaffolds.

The development of zonal release scaffolds and the improvement of cell adhesion may provide the means to enhance simultaneous regeneration of multiple tissues and mimic the natural features of the repair tissue.