Tissue Engineering

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Zonal release of proteins from tissue engineered scaffolds

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This study illustrates the manufacture and characterization of a scaffold for tissue engineering applications, such as peripheral nerve repair where growth factors can be organized into gradients for direction of cell movement, or multi-tissue repair (i.e. osteochondral defects), which will require the development of different tissue types along an interface. Microparticles have been loaded with model proteins, trypsin and horse radish peroxidase (HRP), or recombinant human bone morphogenetic protein-2 (rhBMP-2) using a Solid/Oil/Water emulsion (S/O/W) method as described by Morita et al (2000) and subsequently heat sintered to form a scaffold. An entrapment efficiency of 75% was obtained for the proteins trypsin & HRP using this S/O/W method. HRP was released in a controlled manner from microparticles and scaffolds over a 30-day period and the activity of released protein remained close to 100% of the control throughout the 30-day release period. C2C12 mouse myoblasts were cultured on scaffolds consisting of rhBMP-2loaded and rhBMP-2 free microparticles. After 5 days of culture, alkaline phosphatase (ALP) expression, which is an indicator of differentiation to the bone lineage, was measured. A linear relationship was observed between increasing the ratio of rhBMP-2 loaded microparticles within the scaffold and ALP expression. This demonstrated that cell response to rhBMP-2 is tunable and changing doses of released rhBMP-2 can be achieved by varying the ratio of protein-loaded and protein-free microparticles within the scaffold. Zonal release of protein was established by production of a tri-layered scaffold consisting of a layer of protein-free microparticles sandwiched between two layers of HRP loaded microparticles. The release of HRP was observed through the sequential colour change that occurred when 3,5,3′,5′-tetramethylbenzidine (TMB) substrate was added to the scaffold. The effect of zonal release of rhBMP-2 on the growth of C2C12 mouse myoblasts was also investigated by producing a bi-layered scaffold consisting of a rhBMP-2 loaded zone and a rhBMP-2 free zone. Microscopic visualization following toluidine blue and ALP staining confirmed an even distribution of cells across the scaffold with significantly increased ALP activity in the rhBMP-2 loaded zone.

Morita, T. et al (2000) *J. Controlled Release* **69**: 435–444