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AN EX VIVO BONE CHAMBER FOR BONE TISSUE ENGINEERING

D.B.Jones

Dept. Experimental Orthopaedics and Biomechanics,

Philipps University Marburg, Germany

OVERVIEW: The first publication on the use of mesenchymal stem cells on resorbable scaffolds for bone tissue engineering was in 1987 1. This German patent was based on a series of studies investigating the development of bovine mesenchymal stem cells and small slices of tissue cut from the germanitive zone of the juvenile porcine growth plate grown in the skin pouch of a juvenile nu/nu mouse (2). In the case of the bovine mesenchymal cells innoculation of 1 million cells which had been attached to collagen were scraped off the petri plate an injected under the skin. Figure 1 shows a section of a nodule about 2mm across after 4 weeks. The cells proliferate, but only those within about 100µm of the surface are forming bone. Underneath this bone layer is a zone of vital cartilage, and most of the tissue has is dead. By 6 weeks blood vessels have penetrated the dead cartilaginous tissue and new bone strats to form. The bony nodule is characterized by a disorganized growth. In the case of the 1mm slice of the gorowth plate, after 4 weeks a small rudiment had grown containing epiphyseal cartilage, a growth plate and trabecular bone (figure 2). As in the case of the mesenchymal cell experiment this newly formed bone was of mouse origin, suggesting that with the blood supply new stem cells forming bone were transported to the site. The growth plate and cartilageremained pig tissue however. These results showed that in addition to the growth and differentiation, some organizing factor was required to give shape to the new tissue. However the shape forming material should , if implanted, be able to resorb. We investigated a number of materials and one especially:- poly-l-Lctic acid with varying percentages of tricalcium phosphate (4,5) was investigated closely.

Clearly, in the absence of blood vessels the proposed scaffold should allow a good diffusion path. Krogh's Nobel prize is as applicable now as it ever was. The thickness of trabeculae seem to follow his limit of how far a cell can exist from a surface. In a tissue such as trabecular bone, the number of cells per volume of tissue is far lower than in other tissues, thus the oxygen usage per volume of tissue is low and cells might live by diffusion alone.

In these models an important factor was missing, mechanical forces. Using the flow chamber techniques we developed and adding this to a design for mechanical load we never realized, we built a loading chamber to apply and measure forces and deformations in trabecular bones or scaffolds: the zetos (fig 3). The results of these experiments conducted by us, the AO group of R.Geoff Richards, Everett Smith and Laurence Vico show, in brief, that the response to amplitude and frequency of loading is similar qualitatively to the responses found in vivo.

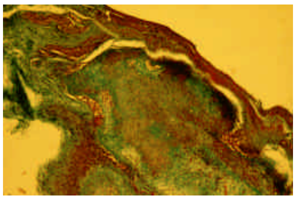

Figure 1

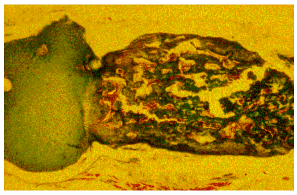

Figure 2

Masson Golder stained tissue grown in the skin pouch of nu/nu mice. Fif. 1 shown after 4 weeks injection of 1 million bovine stem cells. Fig. 2 shows the placement of a 1mm x 1mm x2mm slice of tissue. Both figures show tissue approx 3mm thick at the smallest dimension

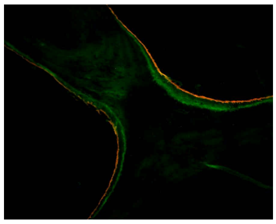

Fig *3. Fluophore staining of bone growth using Rahn's method of human trabecular bone grown in a Zetos chamber*

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