Introduction

Repair of articular cartilage defects is an unresolved clinical problem. (1) Presently there is no universally accepted and satisfactory method for dealing with this commonly encountered medical problem. (2) If cartilage lesions are confined to only articular cartilage there is no cartilage regeneration. If subchondral bone is involved limited repair may occur. Many strategies have been used to deal with these lesions. But all, including autologous chondrocyte transplantation, involve subchondral bone. We have investigated a system whereby hyaline cartilage is formed on flexible demineralized bone which can serve as a subchondral bone plate and thus limit the process of cartilage regeneration to cartilage alone and not to the underlying structures.

Materials and methods

Flexible demineralized bone (Lambone) was prepared from cadaver donors who have met the criteria of acceptance for tissue donation as well as the Rules and Regulations of the USFDA. Bone from the femora or tibiae was sectioned with a non-decalcified bone microtome at less than 1.5mm decalcified with 1N HCL, freeze-dried and subjected to sterilization with irradiation at 2.5 (Mrads). Bone lamina thus prepared produce a chondrogenic response when implanted into experimental animals.

Chondrocytes were derived from cartilage explants which were placed into 75 cm² flasks with DMEM medium with 10% FBS. Multiple cartilage fragments were placed into each flask. Cartilage was obtained from cadaver tissue donors under age 50. Once chondrocytes became confluent they were scraped, placed in suspensions, centrifuged and made into pellets. The pellets were then placed on top of flexible bone preparations also in 75 cm². As an alternative, cells were also seeded on the surface of flexible bone plates (cells seeded in suspensions). (Figures 1 and 2) Histologically cells formed into cohesive structures which had smooth surfaces and grew into irregular surfaces of underlying bone. The cells had features similar to immature chondrocytes. (Fig.3) The matrix produced by the growing cells stained positive with safranin O. (Fig.4) They were also PAS positive.

Results

By two weeks smooth and glistening cartilage clumps became grossly visible. They were either circumscribed formations (seeded as pellets) or covered the entire surface of flexible bone plates (cells seeded in suspensions). (Figures 1 and 2) Histologically cells formed into cohesive structures which had smooth surfaces and grew into irregular surfaces of underlying bone. The cells had features similar to immature chondrocytes. (Fig.3) The matrix produced by the growing cells stained positive with safranin O. (Fig.4) They were also PAS positive.

FIGURES

Fig. 1. Cartilage formed from cell pellet 2 weeks after placement on lambone.

Fig. 2. Cartilage plate on lambone formed from cell suspension two weeks after inoculation.
Discussion

Chondrocyte proliferation which produced tissue compatible with immature hyaline cartilage proceeded rapidly. Cells in culture resembled chondrocytes by their nuclear and cytoplasmic configuration. Many cells contained PAS positive material, most likely glycogen suggesting they were active in producing extracellular matrix. Finally, the performance of the cartilage flexible bone construct had not been evaluated by transplantation into animal models. This must be done before final pronouncement of the technique is made.

References


Disclosures

The author’s have nothing to disclose.