could be expanded in monolayer until passage 5 without loss of their undifferentiated phenotypes.

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SUB-CHONDRAL NACRE IMPLANT IN ARTICULAR ZONE IN SHEEP KNEE

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Purpose: One of the major challenges of orthopedic surgery is the restoration of lost bone and cartilage using biomaterials. Despite recent progress in this area, there is clearly to date no biomaterial than can satisfy all the prerequisites. Mother of pearl (or nacre) is a composite natural biomaterial, source of bioactive molecules having biological activities on cells of the bone lineage and on skin fibroblasts and keratinocytes. This study is designed to identify the behaviour of nacre in subchondral bone area in sheep knee. To this end we followed-up the biointegration of an osteochondral nacreous implant histologically and radiologically.

Methods: We implanted nacre blocks in sheep trochlea, by replacing the half of the femoral trochlea. The subchondral area of the implant was at the level of the sub-chondral bone level not at the cartilage one. For comparison we used complete cartilage resection until sub-chondral bone. After implantation sheep were killed after 3, 6 and 9 months. Histological and radiological evaluations were done on the cartilage/nacre interface without decalcification.

Results: Nacre implants were well tolerated without any synovial reaction. We observed centripetal regrowth of new cartilage in 6/9 cases after 3 months, and radiography showed osteointegration. On the resection sample no cartilage regrowth was observed, but formation of a thin layer of fibrous tissue was noted. After 6 months a new cartilage covered totally the nacre implant. Chondral resurfacing of the endo-articular face was observed after 9 months and cartilaginous islets of hyaline cartilage were observed in deep layers demonstrating the chondroinduction potentialities of nacre.

Conclusions: In conclusion, nacre presents chondroinductive potentialities as sub-chondral implant for cartilage regrowth. The surface of sub-chondral implants of nacre in articular zones was not resorbed, but became covered with fibrocartilaginous tissue that contained islets of hyaline cartilage in the deep layers. Finally this study demonstrates the potential of nacreous biomaterial able to stimulate cartilage repair.

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THE USE OF BONE MATRIX GELATIN AS A TISSUE-INDUCTIVE SCAFFOLD FOR THE IN VITRO CONSTRUCTION OF HUMAN CARTILAGE WITH ADJACENT BONE SEGMENTS

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Purpose: To use Bone Matrix Gelatin (BMG) as a scaffold along with human embryo chondrocytes as seeds for the in vitro tissue engineering of cartilage & bone 'limb' grafts.

Methods: Bone matrix gelatin (BMG) was prepared from cancellous bones of adult rabbits using previously published procedures (Li et al. J Zhejiang Univ Sci B. 2008, 9(1): 22-33). The BMG was cut into a cylinder (5mm wide x 2mm deep) and sterilized with ethylene oxide. Human chondrocytes were isolated from 10th-week human embryo cartilage anlagen by sequential enzyme treatments. One million Passage 2 cells were seeded onto BMG grafts and cultured for 3 weeks in vitro. The culture media was changed every 3 days. After 3 weeks, the engineered tissue was fixed, embedded, and cut into 14μm slices for histological analysis. Slides were stained with hematoxylin & eosin (H&E) and toluidine blue. Immunohistochemical analysis was used to detect the expression of collagen types I, II & X and aggrecan.

Results: H&E staining indicated that the engineered grafts were very similar to an articular cartilage above an epiphyseal growth plate with developing bone in vivo. The tissue most distal to the graft was morphologically similar to bone; i.e. bone trabeculae were observed. The cartilage layer nearest the BMG was thick and exhibited different zonal layers of cells organized similar to that seen in early articular cartilage above a developing growth plate (resting layer, upper and lower proliferative layer and hypertrophic layer). In the deep bone-like trabecula layer, osteoblasts and bone marrow cells were observed. Immunohistochemical analysis indicated that the presence of aggrecan and type II collagen expression in the cartilaginous regions. Type I collagen expression was observed in the deeper bone-like regions and type X collagen expression was observed in the hypertrophic-like zone.

Conclusions: This study has demonstrated that harvesting cells from the proximal cartilaginous anlagen regions of the developing human limb and culturing these with de-mineralized BMG scaffolds can cause a recapitulation of embryonic bone development in vitro; i.e. the potential production tissue engineered articular cartilage, growth plate and bone with trabeculae using in vitro culture systems. These results suggest that cells in the early developing cartilage anlagen are pre-programmed for their phenotypic outcome in limb development and these programming signals are maintained in the in vitro cultures. They also illustrate the potential for using tissue engineering applications for the reconstruction of whole limb segmentss for use in orthopaedic medicine.

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EVALUATION OF ACCELL™ AND LIPOFECTAMINE™ 2000 TO DELIVER SMALL INTERFERING RNA MOLECULES TO FULL-THICKNESS CARTILAGE EXPLANTS

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Purpose: Osteoarthritis (OA) is marked by continual degradation of cartilage and interruption of overall joint homeostasis. Although the in vivo pathogenesis of the disease remains unclear, RNA interference (RNAi) provides a tool that may characterize the molecular causes of OA and offer new treatment tactics. Importantly, RNAi relies as much on successful delivery of small interfering RNA (siRNA) to cells as it does on identification of an effective knockdown sequence. Cartilage poses a unique challenge to in situ delivery of siRNAs given its distinctive architecture and vascular nature. The aim of our study was to identify the transfection reagent that offered the greatest delivery of siRNA molecules to chondrocytes, using both monolayer culture and full-thickness cartilage. Our hypothesis was that Accell™ siRNA, which has been modified for use without additional reagents, would transfect a higher percentage of chondrocytes in intact tissue relative to Lipofectamine™ 2000.

Methods: Chondrocytes, both isolated and in full-thickness cartilage, were harvested from a laboratory animal model of OA and a large animal model noted for its similarity to human cartilage. Monolayer cells and uniform explants were exposed to transfection complexes according to manufacturers’ protocols. Fluorescent