THE USE OF CALCIUM ALGINATE IN THE TREATMENT OF ARTICULAR CARTILAGE DEFECTS

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Introduction: Articular cartilage has only limited capability for intrinsic repair after injury. The use of cells or other therapeutic agents such as growth factors has been suggested to help improve the repair of cartilage. A reliable delivery system for cells or growth factors to the site of injury is needed. In previous studies a variety of biomaterials were tried as carriers for cells or growth factors. In this study we used calcium alginate gel as a delivery vehicle.

Materials and Methods: Bone marrow stromal cells were isolated from bone marrow aspirates from the tibia of a four month old New Zealand White (NZW) rabbit. The cells were cultured in monolayer. Culture medium consisted of Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal bovine serum and 1% ascorbate. Non-adherent cells were discarded after three days in culture. After two weeks in mono-layer culture the cells were lifted using trypsin/EDTA, washed twice in phosphate buffered saline and then suspended in a 1.2% sodium alginate solution. Approximately fifty microliter beads of calcium alginate were formed by exposure of the suspension to 102 mM CaCl2 for ten minutes.

Human recombinant TGF-β was suspended in an aliquot of 1.2% sodium alginate at concentration of 2 µg/ml and 20 ng/ml. Subsequently, calcium alginate beads were formed as described above. 1-1.25 labeled TGF-β was used to determine the release rates of polypeptide from the alginate gel in vitro. Counts per minute released into standard culture media were measured at regular intervals over five days using a gamma counter.

Osteochondral defects measuring four millimeters in diameter were created in the trochlear grooves of forty-four-month-old NZW rabbits. Animals were randomly assigned to five different treatment groups. The osteochondral defects were treated with either plain alginate, alginate containing marrow stromal cells at a density of 10⁷ cells per milliliter or with alginate containing TGF-β either at 20 ng/ml or 20 µg/ml. Untreated defects served as control.

Animals were euthanized after six and twelve weeks. The knees were dissected and the repair grossly evaluated using a modified ten point grading scale. The specimens were fixed, decalcified and mounted in paraffin. Histological sections were stained with Safranin O and evaluated using a twenty-four point histological grading scale. Specimens were graded by two independent, blinded observers. Mean scores and standard deviations for each treatment group were calculated. Probability values were determined using the student’s t-test.

Results: TGF-beta was released from the calcium alginate beads at a slow, constant rate that was sustained throughout the time of observation. After five days the amount of TGF-beta released into the culture media for the beads containing the 1ug/ml and 10 ug/ml of growth factor was thirty and forty percent, respectively (Fig.1). All animals tolerated the surgical procedure well and no complications were encountered. Without treatment 60 percent of the defects were filled with repair tissue at six weeks and 75% percent at twelve weeks. The use of calcium alginate increased these rates to 70 (p=0.05) and 100 percent (p=0.05), respectively. The use of TGF-β led to increased osteophyte formation and exuberant repair tissue in the defects. This effect was dose-dependent. Treatment with cells in combination with alginate resulted in a delay of the joint surface restoration at 6 weeks, but at twelve weeks this effect had disappeared and on the 10 point gross grading scale this group achieved the highest scores. The mean score was 9.4 compared with a score of 8.3 for the control defects (p<0.02).

Histological evaluation of the repair tissues demonstrated an advantage for defects treated with alginate (score=16.4) compared to control specimens (score=13.6) at six weeks. This difference was statistically significant (p<0.007). However, at twelve weeks no statistically significant differences were observed.

Conclusions: Our results indicate that the use of alginate may improve the repair of osteochondral defects in the rabbit model as shown by the significant improvement of the repair after six weeks. The combination of bone marrow stromal cells with alginate showed very promising results after twelve weeks. Longer term follow up studies are needed to investigate whether the beneficial effects of the alginate can be maintained. The use of TGF-β was associated with increased osteophyte formation in a dose-dependent fashion. At lower concentrations it is possible that TGF-β may accelerate the restoration of the joint surface without the detrimental side effects. Alginate allows the controlled delivery of therapeutic agents such as growth factors or cells to articular cartilage defects. The results of our in vivo study demonstrate improved defect filling with alginate plus stromal cells compared to TGF-β, alginate alone, or untreated defects.

The in vitro study of the release kinetics of TGF-β from the alginate demonstrate a slow, sustained release of the polypeptide factor. This indicates that alginate may serve for the sustained, local delivery of growth factors and other therapeutic agents at the site of injury.

Figure 1: Release of TGF-β into the culture media measured as counts per minute plotted against time.

Reference: Britberg et al., Clinical Orthopaedics and Related Research, 326, pp 270-283