Implantation of Autologous Chondrocytes in a 3-D Resorbable Polymer Fleece for the Treatment of Cartilage Defects in the Knee Joint

C. Erggelet, A. Dauch, A. Lahm, P. Kreuz, C. Ossendorf, E. Mrosek and M. Sittinger*

University of Freiburg, Germany
**Charite, Berlin, Germany*

Introduction

Damaged or diseased articular cartilage frequently leads to progressive debilitation resulting in a marked decrease in the quality of life. Tissue engineering, a budding field in modern biomedical sciences, promises creation of viable substitutes for failing organs or tissues. Current tissue engineering approaches are mainly focused on the restoration of pathologically altered tissue structure based on the transplantation of cells in combination with supportive matrices and biomolecules.

Chondrocytes undergo a process of phenotypic and functional dedifferentiation when cultured in monolayer systems that lack the crucial influence of physiological cell–cell and cell–extracellular matrix (ECM) interactions. A growing body of evidence indicates that these interactions, which directly influence cell signalling via cell adhesion molecules, are of vital importance for nearly all cell functions. 3D cell cultures provide the advantage of anchorage independent cell growth allowing cell motility, the synthesis of a specific pericellular or intercellular matrix and the physiological release and storage of bioactive molecules such as cytokines and morphogenic factors. Essential components of cartilage tissue engineering

Autologous Chondrocytes

Tissue engineering protocols usually require handling of isolated autologous cells. Tissue samples from patients have to be isolated by

enzymes such as collagenase and hyaluronidase to remove extracellular matrix components. All the subsequent steps have to be carefully executed to avoid contamination or potential infections by media and supplements. So far, most approaches to tissue repair by autologous cells use biopsies from healthy sites on contra lateral tissues. For a successful transfer into clinics, two major goals have to be achieved: (1) a simple and minimal invasive procedure to collect cells from the patient and (2) differentiation of crucial functional properties (e.g. mechanical stability) *in vitro* or *in vivo* within a short time.

Scaffolds for Tissue Construction

Specially designed biomaterial scaffolds are one of the key components in tissue engineering. Research is focused on developing bioresorbable scaffolds that exhibit optimal physical properties coupled with excellent biocompatibility. Scaffolds act as shape and guidance templates for *in vitro* and *in vivo* tissue development. For cartilage and bone tissues, a suitable scaffold provides initial mechanical stability and supports even cell distribution. Natural polymeric gels, such as hyaluronic acid, collagen, alginate and chitosan, have been used successfully. These scaffolds permit 3D immobilization of cells and maintain the differentiated phenotype of chondrocytes. However, their mechanical behaviour is insufficient for tissue transplantation and so solid bioresorbable fiber scaffolds or other porous structures are used to achieve initial biomechanical stability. Synthetic biodegradable poly-alpha-hydroxy esters such as polylactic acid (PLLA), polyglycolic acid (PGA) and copolymer PLGA have been used extensively in this context. Both types of materials increase proteoglycan synthesis compared with collagen scaffolds. Injectable *in situ* crosslinkable polymeric preparations that entrap cells have been designed and techniques that combine the advantages of both porous fibre structures and gels are being explored as suitable alternatives to either gels or fibre scaffolds.

Bioreactors

Although our understanding of cell biology has increased enormously in recent years, the methods of handling *in vitro* cultures of human cells have hardly changed. As demonstrated recently, the ability of conventional monolayer cultures to generate highly differentiated structures is limited because cells are cultured on an inappropriate substrate owing to lack of the requisite characteristic extracellular matrix environment. Moreover, metabolic conditions in the culture medium fluctuate and high density, long-term cultures are always at a risk of contamination. To address the aforementioned problems artificial tissue constructs can be cultured in rotating bioreactor vessels or perfusion culture systems. Another major problem, which has to

be solved, is the fixation of the cartilage transplant to the subchondral bone in the joint. In theory, the artificially grown cartilage layers could be attached directly to the defect joint surface using fibrin glue, or it could be fixed using resorbable pins. The ultimate aim is to achieve a permanent, solid connection between cartilage and bone tissue. Autologous chondrocyte implantation (ACI) is considered to be an established option for the clinical application of tissue engineering for the treatment of cartilage defects in the knee. To overcome technical limitations regarding intra-operative handling, initial biomechanical stability and standardized cell distribution a prospective clinical pilot study was inaugurated in October 2001 using a resorbable 3-D polymer fleece as a carrier system for autologous chondrocytes.

Materials and Methods

45 patients were operated with ACI between 10/2001 and 11/.2002. 42 patients were available for follow-up. 20 female and 25 male patients, aged 39 years with an av. BMI of 34,57, had 3,3 previous cartilage related surgery prior to biopsy for ACI. The av. defect size was 3,98 cm^2 mainly on the MFC. Pre-op and at 3,6 and 12 months post ACI a mod. Cincinnati knee rating scale, ICRS, KOOS, SF36 and MRI were evaluated.

The technique is based on a 2 mm thick polymer fleece (polyglactin/poly-p-dioxanon) loaded with 5 $x10^6$ /cm^2 autologous chondrocytes in a fibrin gel matrix which is transosseously fixated and therefore allows coverage of large lesions even with incomplete containment.

The defect site is debrided to a rectangular shape down to the subchon-

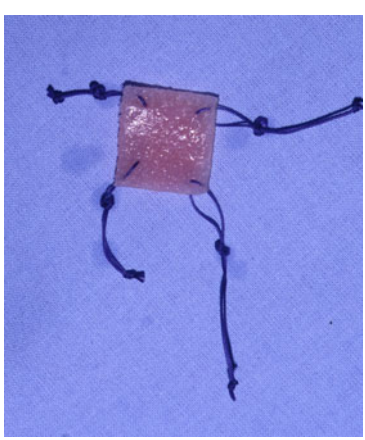

Fig 1 Polymer fleece seeded with autolgous chondrocytes armed with threads (Vicryl 2-0) prior to transosseous fixation

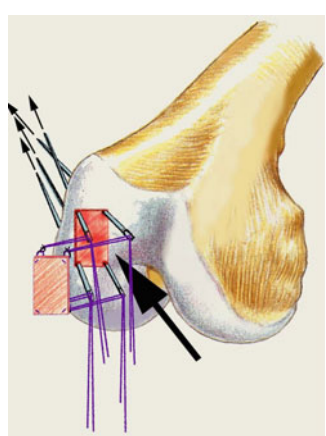

Fig 2 Implantation and transosseous fixation of a polymer fleece seeded with autolgous chondrocytes (Illustration)

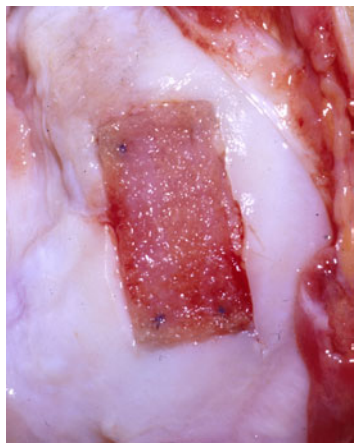

Fig 3 Polymer fleece seeded with autolgous chondrocytes implanted in cartilage defecton the femoral condyle

dral bone. A stable shoulder surrounding the defect has to be preferred. The defect size is then determined and the construct cut accordingly. All four corners of the defect are drilled with a guide wire in an inside - out technique. Using a resorbable thread (Vicryl 2-0) the scaffold is armed on the corners. One three-fold knot approx. 1 cm from the edge secures the sling. An additional knot approx. 2 cm out moores the sling which serves as a pulley (fig 1). The pulley slings are now pulled into the joint by the guide wire and through the femoral bone (fig 2). Firm action on the pulleys guides the knots into the drill holes. The scaffold is now securely anchored (fig 3). The pulley slings are cut close to their dermal exit and removed.

Results

At 1 year the mean Cincinnati score had increased from 3.58 to 5.88. 2 patients had not improved. 9 patients underwent re-arthroscopy. 3 patients agreed on taking a biopsy (fig 4). 1 patient developed an acute bacterial arthritis, 1 patient showed persistent effusion until 6 months post operatively. No other specific adverse events were seen.

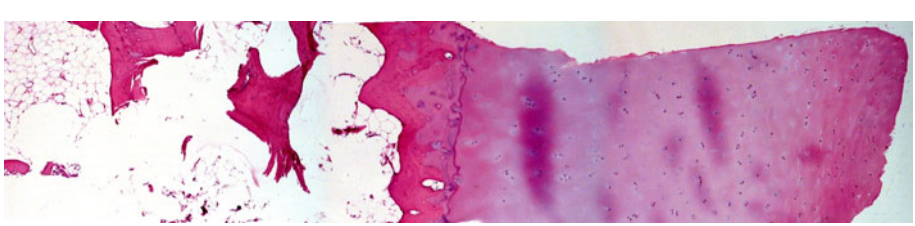

Fig 4 Core biopsy 9 months after ACI with polymer-fleece

Conclusions

The key to successful repair and regeneration of cartilage is to provide the repair site with sufficient chondrogenic cells in a suitable delivery vehicle to ensure maximal differentiation and deposition of right extracellular matrix. New optimized culture methodologies and bioreactors that provideappropriate mechanical and other guidance clues must be engineered to ensure the successful function of engineered tissue. As we gain more and more information about the identity of all of the morphogens for chondrocyte differentiation it might be possible to orchestrate massive cartilage regeneration by clever combination of smart 3D scaffolds and such morphogenic factors. The clinical use of resorbable carrier systems for autologous chondrocytes shows a significant improvement of the intraoperative handling and a short term outcome comparable to the standard technique.

References

Barnewitz D, Evers A, Zimmermann J, Wilke I, Kaps C, Sittinger M. Tissue engineering: new treatment of cartilage alterations in degenerative joint diseases in horses—preliminary results of a long term studyBerl Munch Tierarztl Wochenschr. 2003 Mar-Apr;116(3-4):157-61.

Erggelet C, Sittinger M, Lahm A. The arthroscopic implantation of autologous chondrocytes for the treatment of full-thickness cartilage defects of the knee joint. Arthroscopy. 2003 Jan;19(1):108-10.

Perka C, Schultz O, Sittinger M, Zippel H. Chondrocyte transplantation in PGLA/polydioxanone fleece Orthopade. 2000 Feb;29(2):112-9.

Risbud MV, Sittinger M. Tissue engineering: advances in in vitro cartilage generation. Trends Biotechnol. 2002 Aug;20(8):351-6.