Repair of human articular cartilage after implantation of autologous chondrocytes

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Tissue engineering is an increasingly popular method of addressing pathological disorders of cartilage. Recent studies have demonstrated its clinical efficacy, but there is little information on the structural organisation and biochemical composition of the repair tissue and its relation to the adjacent normal tissue. We therefore analysed by polarised light microscopy and immunohistochemistry biopsies of repair tissue which had been taken 12 months after implantation of autologous chondrocytes in two patients with defects of articular cartilage.

Our findings showed zonal heterogeneity throughout the repair tissue. The deeper zone resembled hyaline-like articular cartilage whereas the upper zone was more fibrocartilaginous. The results indicate that within 12 months autologous chondrocyte implantation successfully produces replacement cartilage tissue, a major part of which resembles normal hyaline cartilage.

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It has long been known that the intrinsic ability of articular cartilage to repair damage is severely limited.1,2 While small defects may cause no clinical problem, larger areas may result in pain, swelling and possibly progression to osteoarthritis. In recent years there has been much interest and research into mechanisms which may facilitate repair of articular cartilage such as debridement,3 drilling of subchondral bone4 and periosteal and perichondrial transplantation.5,6 Repair tissue may form after many of these treatments, but it often resembles fibrocartilage or lacks cohesive attachment to the underlying bone.6 Autologous chondrocyte implantation (ACI) is becoming a widely used technique in Sweden, Germany and the USA for treating full-depth osteochondral defects of articular cartilage.7 The clinical follow-up is encouraging8 in spite of the initial scepticism. The outcome of such treatments has been monitored by clinical and functional assessments, but little is known of the structural and biological organisation of the repair tissue.

We have therefore undertaken a detailed immunohistochemical analysis of biopsies of repair tissue from two patients one year after ACI.

Patients and Methods

Two patients, aged 28 and 32 years, presented with painful knees and defects of the medial femoral condyles covering areas of 4 cm2 and 2 cm2, respectively. We removed cartilage from a non-weight-bearing surface of the affected knee at arthroscopy from which chondrocytes were isolated and cultured in the laboratory (Carticel; Genzyme Tissue Repair, Cambridge, Massachusetts). Four to five weeks later the defect was trimmed back to healthy cartilage, squared off and curetted down through the calcified layer towards the bone resulting in minimal bleeding. A periosteal flap, taken from the proximal medial tibia, was sutured over the defect and the cultured chondrocytes (approximately 12 × 106) were injected.7

Twelve months after the operation functional assessment of the repair was undertaken and a full-depth core biopsy, 2 mm in diameter, was obtained at a further arthroscopy. This tissue was snap-frozen and examined by routine histology, polarised light microscopy and immunohistochemistry for collagen and proteoglycan components. Antibodies to collagens I, III, VI and X were kindly provided by Drs V. Duance, Cardiff, UK (types I and III), S. Ayad, Manchester, UK (type VI), G. Gibson, Detroit, USA (type X) and A. Kwan, Cardiff, UK (type X) while those to type-II collagen were obtained from the Developmental Studies Hybridoma Bank, USA (CIIIC1). In addition, various proteoglycan components were investigated using antibodies produced and characterised in our laboratories. These included glycosaminoglycans, keratan sulphate (antibody 5D4), chondroitin-4-sulphate (2B6) and chondroitin-6-sulphate
(3B3+/-, 7D4), link protein (8A4) and hyaluronic acid-binding region (1C6). All antibodies were monoclonal except those to type-I, type-III and type-VI collagen which were polyclonal. Frozen sections 6 µm thick were fixed in 10% formalin in phosphate-buffered saline (PBS). Some sections were predigested with hyaluronidase or chondroitinase ABC to facilitate the exposure of collagen and proteoglycan epitopes, as described previously. Sections incubated with monoclonal antibodies were subsequently labelled with a biotinylated secondary antibody, the endogenous peroxidase activity being blocked with 0.3% hydrogen peroxide in methanol before labelling with a peroxidase-labelled biotin-streptavidin complex (Vectastain Elite; Vector Laboratories, Peterborough, UK) and diaminobenzidine. A similar procedure was used for polyclonal antibodies but without the biotin-streptavidin amplification. Adjacent sections were incubated with PBS or an inappropriate antibody (e.g., anti-soya bean, kindly provided by Dr V. Duance, Cardiff) in place of the primary antibody (e.g., anti-soya bean, kindly provided by Dr V. Duance, Cardiff) in place of the primary antibody as controls. For comparison, tissue was also incubated with monoclonal antibodies but without the biotin-streptavidin amplification. Adjacent sections were incubated with PBS or an inappropriate antibody (e.g., anti-soya bean, kindly provided by Dr V. Duance, Cardiff) in place of the primary antibody as controls. For comparison, tissue was also incubated with monoclonal antibodies but without the biotin-streptavidin amplification.

Results

Both the ACI-treated patients showed symptomatic improvement at one year after implantation, with Lysholm scores raising from 68 to 76 and 39 to 56, respectively. Arthroscopy showed one graft to be firm and the other soft. Both were higher than the surrounding tissue but integrated well at the margins. The biopsies showed excellent integration with the underlying bone. There was an intermediate layer of calcified cartilage typical of that found in normal adult articular cartilage (Fig. 1). The lower portion of the biopsies was morphologically indistinguishable from normal tissue. By contrast, the upper region had a fibrocartilaginous appearance with collagen fibres arranged less regularly (Fig. 1c), apart from in the superficial layer where they were aligned parallel to the surface.

The immunostaining of the deeper zone was typical of hyaline cartilage, being positive for type-II collagen throughout the matrix (Figs 2a to 2d) with predominantly pericellular staining for type-VI collagen and similar to that of a non-grafted joint from a 13-year-old boy (2e). Type-I and type-III collagen were seen particularly in the upper region (Fig. 2i). Type-X collagen was observed around the cells in the hyaline-like cartilage but rarely in the upper zone (Figs 2f and 2g). Staining for keratan- and chondroitin-4- and chondroitin-6-sulphate also occurred; the strongest staining for both chondroitin-4- and chondroitin-6-sulphate (2B6 and 3B3(+)), respectively was throughout the matrix in the fibrocartilaginous tissue but was pericellular in the hyaline-like cartilage (Figs 2h and 2j). There was also sparse staining for the epitopes identified as 3B3(-) and 7D4. For 3B3(-) this occurred in the upper region but not in the lower part and vice versa for 7D4. These epitopes typically occur in developing articular cartilage, the growth plate and repairing osteoarthritic cartilage. In some sections of the biopsies the hyaline-like and fibrocartilage-like regions were clearly demarcated (Fig. 1). In others there were numerous small islands within the fibrocartilaginous region which had the appearance of hyaline cartilage (Figs 2h and 2i).

Discussion

Biopsies of repair tissue have been studied previously but the detailed macromolecular species present and their manner of organisation have not been investigated. Our study shows vertical orientation of collagen in the deep zone which is integrated with the underlying calcified layer of cartilage, suggesting successful replacement of tissue in this region. Similarly, the orientation of the collagen fibres which lie parallel to the articulating surface in the surface zone resembles that found in normal joints. The origin of this layer, however, remains unknown. It is possible that it may have been derived from or be vestigial periosteum. Alternatively, it could be repair cartilage produced by the implanted chondrocytes. The role of the periostium requires clarification as to whether it is simply a mechanical restraint for the grafted chondrocytes or whether it provides factors and/or cells which may influence the repair process.

Both the morphological characteristics and the immunohistochemical analyses indicate that the deeper zone was predominantly composed of matrix components similar to those seen in normal articular cartilage, as shown by the presence of type-II collagen throughout, type-VI collagen pericellularly, chondroitin-6-sulphate (3B3 epitope) and keratan sulphate. The upper zone, in contrast, was more fibrocartilaginous with more type-I and type-III collagen than type-II collagen and glycosaminoglycans typical of those found in the meniscus (chondroitin-4-sulphate (2B6) and chondroitin-6-sulphate (3B3(-))).

Type-X collagen was first isolated from hypertrophic chondrocytes in epiphysal plates and was thought to play a pivotal role in the subsequent mineralisation of the matrix tissues. More recently, however, it has been found in normal articular cartilage and also in intervertebral discs. It is considered to be a marker for a change in the chondrocyte phenotype to a hypertrophic state. The exact function of this collagen remains obscure, but it is suggested that it may exert other influences on the extracellular matrix such as altering the accumulation of proteoglycans, thereby modifying the diffusion properties of the matrix. In addition, it has the potential of being more susceptible to digestion by collagenase enzymes, making it a candidate for relatively easy turnover. The role of type-X collagen in the biopsy samples is unclear. It could indicate an active process of endochondral ossification leading to integration of the newly-formed articular cartilage with the subchondral bone, or alternatively, it may be indicative of cartilage remodelling and provide an interim
Figure 1a – Photomicrograph showing a full-depth core biopsy of repair tissue taken 12 months after implantation in a 32-year-old man (haematoxylin and eosin; bar = 500 µ). Figures 1b and 1d – At a higher magnification showing b) upper and d) mid and deep zones (bars = 100 µ). Figures 1c and 1e – The same sections viewed under polarised light. There is parallel alignment (arrow) of collagen fibres in the superficial layer (c), below which the collagen fibres are random. In the lower part of the biopsy (e) the collagen is arranged more perpendicular to the underlying bone (F, fibrocartilage-like tissue; H, hyaline-like tissue; CC, calcified cartilage; B, subchondral bone).
Immunostaining demonstrates the occurrence of type-II collagen predominantly in the hyaline-like cartilage (H) and the calcified cartilage (CC), but less in the upper, fibrocartilaginous matrix (F) and none in the bone (B) in ACI-treated patients (a to d). In the lower part of the biopsy (d) and when viewed under polarised light (c) there is exactly the same distribution of immunostaining for type-II collagen as in cartilage from a 13-year-old boy (e). Staining for type-X collagen occurred in the interterritorial matrix around the cells of the deep zone in the 28-year-old patient who had ACI (f). In the sample from the non-grafted joint of a 13-year-old boy (g), however, type-X collagen was restricted to a narrow band in the region of the tidemark. Islands of matrix within the fibrocartilaginous tissue had the immunohistochemical appearance of hyaline-like cartilage, with less staining for chondroitin-4-sulphate (h, 2B6, arrow) and type-I collagen (i, arrows) than the surrounding matrix. Immunostaining with 5D4 antibody showed keratan sulphate in hyaline-like cartilage (j) (bars = 100 µ) (h, i, 52-year-old ACI patient; j, 28-year-old ACI patient).
collagen scaffold, as demonstrated in the growth plate. The presence of 3B3(-) and 7D4 epitopes on cartilage proteoglycans is considered to be an indicator of remodelling within the cartilage. These biopsies provide only a ‘snap-shot’ of the dynamic events which occur in the repair process of cartilage but show that at 12 months the repair tissue is heterogenous with respect to the matrix components and their organisation. None the less, our analysis showed a considerably higher proportion of hyaline-like cartilage than the relatively small amount (2%) recently reported in failed ACIs.

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