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A hydrophylic polymer system enhanced articular cartilage regeneration *in vivo*

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This study describes a new method for the repair of large articular cartilage defects in the knee joint and compares the effect of two polymer systems on the quality of the repair tissue. The two systems are a newly developed hydrophylic system, based on poly-ethyl-methacrylate (PEMA) polymer and tetra-hydro-furfuryl-methacrylate (THFMA) monomer and the conventional bone cement polymer system, based on poly-methyl-methacrylate (PMMA) polymer and methyl-methacrylate (MMA) monomer. Thirty adult Sandy-lop rabbits were used. Both knees were operated on in each animal, the one defect received either PEMA/THFMA or conventional bone cement and the contralateral defect received no biomaterial (control group). Femora were retrieved at six weeks and the repair tissue was studied by histology, histochemistry and immuno-histochemistry. PEMA/THFMA enhanced the quality of the repair significantly ($p < 0.0001$). By six weeks hyaline-like articular cartilage was the predominant tissue covering the defects and it was fully integrated with the surrounding normal articular cartilage. Immuno-localization showed cartilage components, including collagen type II, distributed evenly throughout its matrix. PMMA/MMA on the other hand did not improve significantly the repair tissue, which was predominately fibro-cartilaginous, poorly bonded to the adjacent normal articular cartilage. The method of implantation is simple and easily reproducible and the new polymer has been well-accepted by the rabbits.

1. Introduction

The articular cartilage repair response has been the focus of investigations for many years but still the quality of the repair tissue, following a localized full-thickness injury to the cartilage is unpredictable. Moreover, patients with focal articular cartilage lesions are, in the vast majority, young and productive and therefore, these injuries present serious social and financial implications.

Biomaterial scientists continue to search for a bioactive material that enhances the biological repair process following injury to the articular cartilage. To date, materials such as carbon fibre [1, 2], hydrogels [3] or polyglycolic/polylactic acid [4], with or without chondrocytes have been tried with various levels of success. In previous studies [5, 6], it has been shown that a newly developed hydrophylic polymer system, based on poly-ethyl-methacrylate (PEMA) polymer powder and tetra-hydro-furfuryl-methacrylate (THFMA) monomer liquid, improves significantly the quality of repair in large, full-thickness articular cartilage defects. Based on those findings, the present study utilizes PEMA/THFMA and conventional bone cement, based on poly-methyl-methacrylate (PMMA) polymer powder and methyl-methacrylate (MMA) liquid. The two polymers were implanted individually in the same way in large articular cartilage defects and their effect on the quality of the repair tissue was compared, to clarify, among others, whether the method of implantation matters mostly, or whether the chemistry of the new polymer is also critical.

2. Materials and Methods

2.1. Animal model

Thirty adult, female Sandy-lop rabbits were anaesthetized with 2% Halothane and a 3:2 mixture of nitrous oxide and oxygen. The knee joint was approached through a medial parapatellar approach and the articular cartilage defect was created by hand, using a 4.5 mm drill bit. All osteochondral debris were thoroughly washed with normal saline prior to polymer implantation.

Preparation of the polymer systems was made at room temperature using a sterile spatula. For the PEMA/THFMA system, 1 gr PEMA (containing 8%

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Barium Sulphate) was mixed with 0.6 ml THFMA, while for the PMMA/MMA system, 1 gr PMMA (containing 10% Barium Sulphate) was mixed with 0.5 ml MMA. Both monomer liquid preparations contained 2.5% V/V N,N-dimethyl-p-toluidine. Using a sterile syringe, 0.1 ml of either dough was injected into the defects. Polymerization occurred *in situ*. In most cases bleeding from the defects was minimal, not interfering with accurate positioning of the polymer plugs. During polymerization, using the flat end of a 4.5 mm wide cylindrical rod, constant pressure was applied at the top surface of the plugs, thus carefully controlling the level of implantation into the subchondral bone, 2 mm below the level of the adjacent articular cartilage.

In this study, osteochondral defects were created in the femoral trochlea, opposite to the articulating patella, which is a highly loaded area of the knee joint. The rabbits were group-housed in floor-pens [7] to allow complete freedom of movements as well as full weight-bearing both pre- and post-operatively. Animals were killed at 6 weeks post-operatively and the repair tissue was studied by histology, histochemistry and immuno-histochemistry.

2.2. Preparation for histology–histochemistry

Distal femora, including the resurfaced defect area, were fixed in 4% paraformaldehyde and 0.3% glutaraldehyde in 0.1 M sodium cacodylate for 48 h. They decalcified with neutral EDTA until no calcium could be detected by radiography. After decalcification samples were washed in 0.1 M sodium cacodylate buffer for a further 24 h and dehydrated through a gradual series of ethanols and xylene for 24 h at room temperature. Samples were then impregnated into wax at 60 °C, before embedding into wax blocks. Sagittal sections, 5 microns thick, including the whole of the repair tissue and the adjacent to the defect normal articular cartilage were cut and stained with haematoxylin/eosin and safranin-O stains.

2.3. Preparation for immuno–histochemistry

The newly developed silver-enhanced colloidal gold immuno-staining was applied, the proprietary kit was provided by Bioclin (Cardiff, Wales). Four selected monoclonal antibodies were applied individually: the anti-type II collagen antibody (CIICI, Development Studies Hybridoma Bank, Iowa, USA, dilution 1:1), the anti-keratan sulphate (5D4, ICN Biomedicals, Bucks, UK, dilution 1:500), the anti-chondroitin 4-sulphate antibody (2B6, ICN Biomedicals, Bucks, UK, dilution 1:100) and the anti-chondroitin 6-sulphate antibody (3B3, ICN Biomedicals, Bucks, UK, dilution 1:100).

Sections were dewaxed in xylene, rehydrated through graded series of ethanols and incubated with hyaluronidase (10 IU/ml, Sigma, Poole, UK) and chondroitinase ABC (0.25 IU/ml, Sigma, Poole, UK) for 2 h at room temperature. Incubation with the primary antibody was made overnight at 4° C in a dark, humidified atmosphere. Antibodies were diluted individually in phosphate-buffer-saline with 0.6% bovine serum albumin (Sigma, Poole, UK). Sections were incubated with the colloidal gold conjugated anti-mouse Ig secondary antibody, dilution 1:50, for 2 h at room temperature and fixed in 1% glutaraldehyde. Colloidal gold conjugates were visualized by use of a physical silver development solution and sections were counter-stained with Mayer's haematoxyline.

3. Results

No infection was recorded in any rabbit and there was no inflammatory or symptomatic foreign-body reaction noted in the synovium samples, taken from all operated knees.

Twelve (80%) of the defects filled with PEMA/THFMA were fully resurfaced by six weeks with hyaline-like articular cartilage, fully integrated with the surrounding the defect normal articular cartilage (Fig. 1). The reparative tissue contained numerous cells expressing chondrocyte phenotype. These cells were mainly distributed throughout the middle and deep layers of the newly formed cartilage, while the superficial zone contained small and flat cells, lying parallel to the articular surface (Fig. 2). All but one specimens staining with safranin-O, showed normal or near to normal concentration of proteoglycans in the repair tissue compared to the adjacent normal articular cartilage. Moreover, a zonal differentiation between the light staining of the superficial layer compared to the deep layers was found in all specimens in the PEMA/THFMA group (Fig. 3). In the same group, immuno-histochemistry detected cartilage components, including collagen type II, keratan sulphate, and chondroitin 4- and 6-sulphate, evenly distributed in the matrix of the reparative tissue in all twelve fully resurfaced knees (Fig. 4).

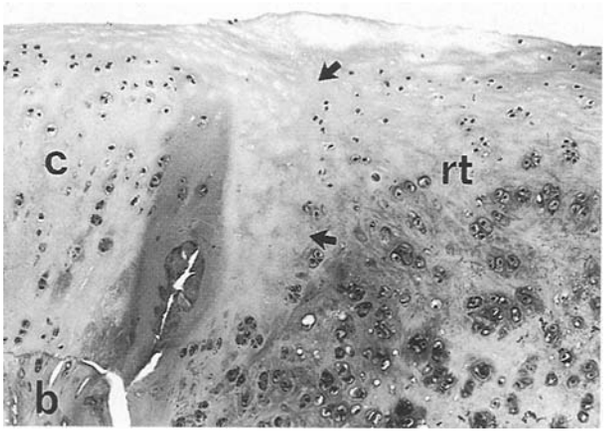

Figure 1 Junction between repair tissue and normal articular cartilage in the PEMA/THFMA group. The defect has been resurfaced with hyaline-like articular cartilage, which is fully integrated with the adjacent normal articular cartilage. (rt = repair tissue, c = normal articular cartilage, b = subchondral bone, the arrows show the complete integration of the new with the old cartilage). [Haematoxylin-Eosin staining, x 15]

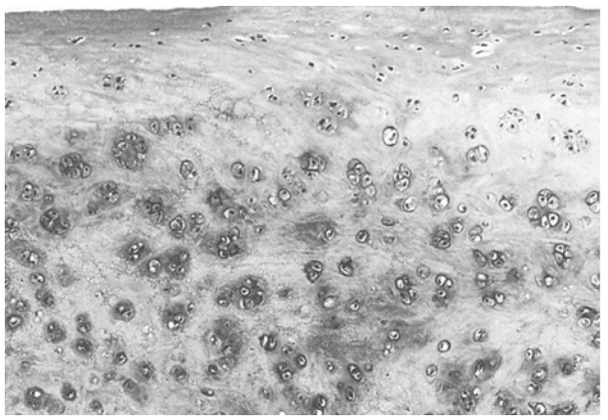

Figure 2 Repair tissue in the PEMA/THFMA group. Numerous chondrocytes can be seen in the middle and deep layers of the newly formed articular cartilage and its surface is smooth and intact. [Haematoxylin-Eosin staining, x 15]

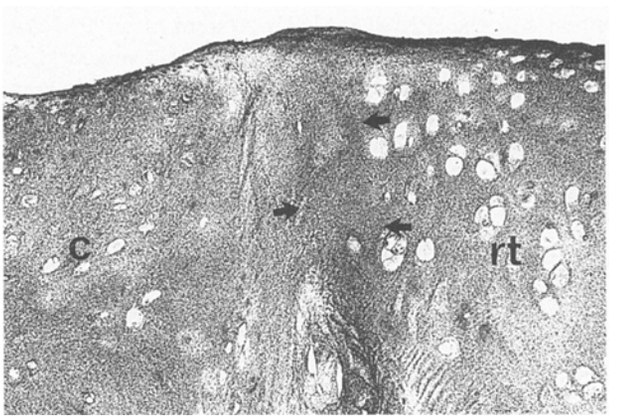

Figure 4 Junction between repair tissue and normal articular cartilage in the PEMA/THFMA group. Immuno-histochemistry shows collagen type II evenly distributed throughout the matrix of the newly formed cartilage. (rt = repair tissue, c = normal articular cartilage, the arrows show the junction between the new and the old cartilage that contains significant amount of collagen type II). [Silver-enhanced colloidal-gold staining, x 15]

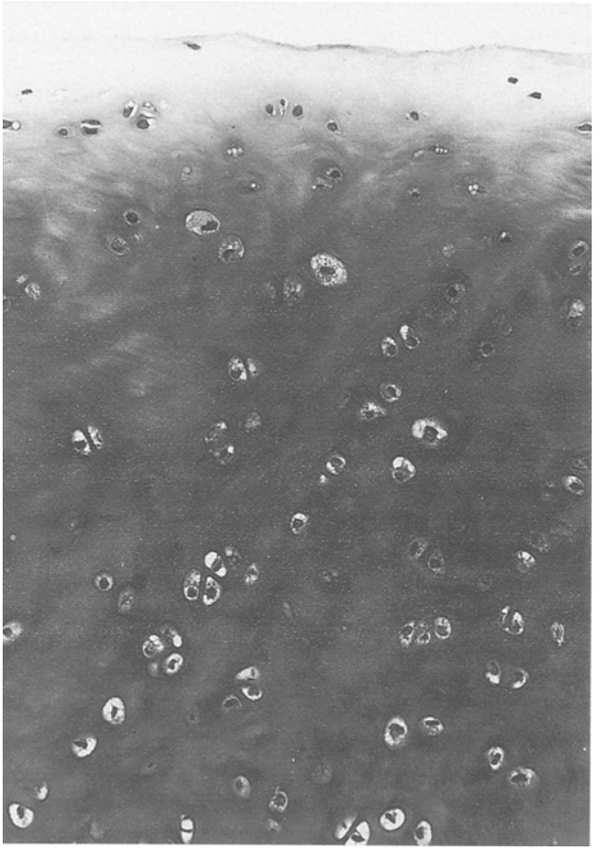

Figure 3 The matrix of the newly formed articular cartilage in the PEMA/ THFMA group contains proteoglycans in high concentration and shows zonal differentiation between the superficial and the deep layers, a characteristic of mature cartilage. [Safranin-O staining, x 20]

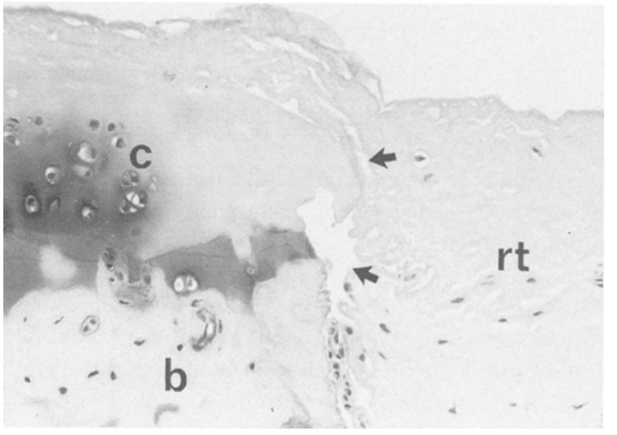

Figure 5 Junction between repair tissue and normal articular cartilage in the PMMA/MMA group. The repair tissue is fibrocartilage incompletely bonded to the adjacent " normal " articular cartilage, which at the edge is acellular and deformed. (rt = repair tissue, c = normal articular cartilage, b = subchondral bone, the arrows show the incomplete bonding). [Haematoxylin-Eosin staining, x 15]

Of the defects implanted with PMMA/MMA, all were repaired with predominately fibrocartilaginous type of tissue, with poor structural characteristics and incomplete bonding to the surrounding normal articular cartilage. The latter presented with a characteristic acellular edge and degenerative changes, mainly affecting the superficial layer, in all specimens (Fig. 5). Histochemistry showed proteoglycans in the deep layer of the repair tissue only and no zonal differentiation (Fig. 6) and immuno–histochemistry detected collagen type II in five specimens only, distributed in the deep zone of the reparative tissue. In the control group, 77% of the defects were incompletely resurfaced with fibroblastic tissue, poorly bonded to normal articular cartilage (Fig. 7). Immuno-staining showed proteoglycan aggregations, but no collagen type II, located exclusively pericellularly, only in the deeper layers.

4. Discussion

The present *in vivo* study compared the new hydrophylic polymer PEMA/ THFMA to the conventional bone cement acrylic polymer as biomaterials to enhance articular cartilage repair, in large, full-thickness defects, in the knee joint. PEMA/THFMA was previously shown to have excellent biological properties in the oral mucosa [8] as well as following

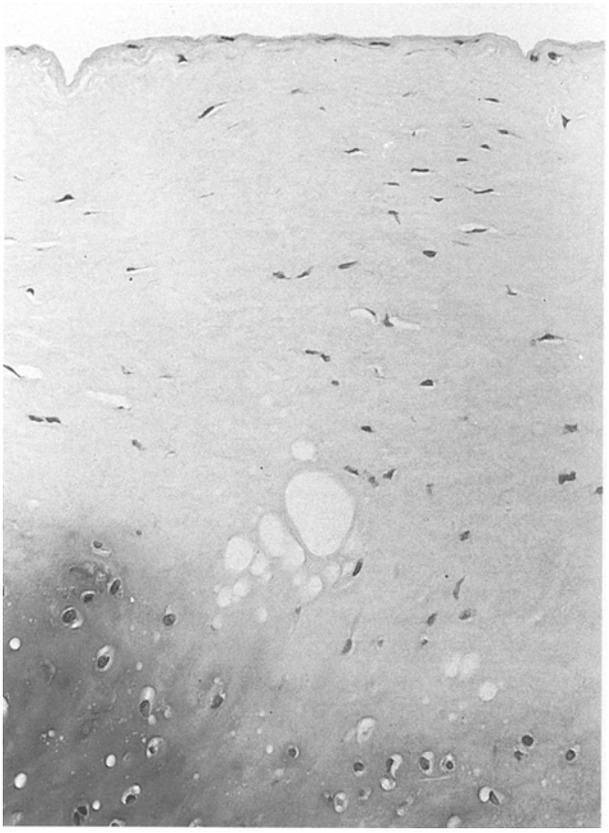

Figure 6 The matrix of the repair tissue in the PMMA/MMA group contains no proteoglycans except in few areas in the deep layer only. [Safranin-O staining, x 20]

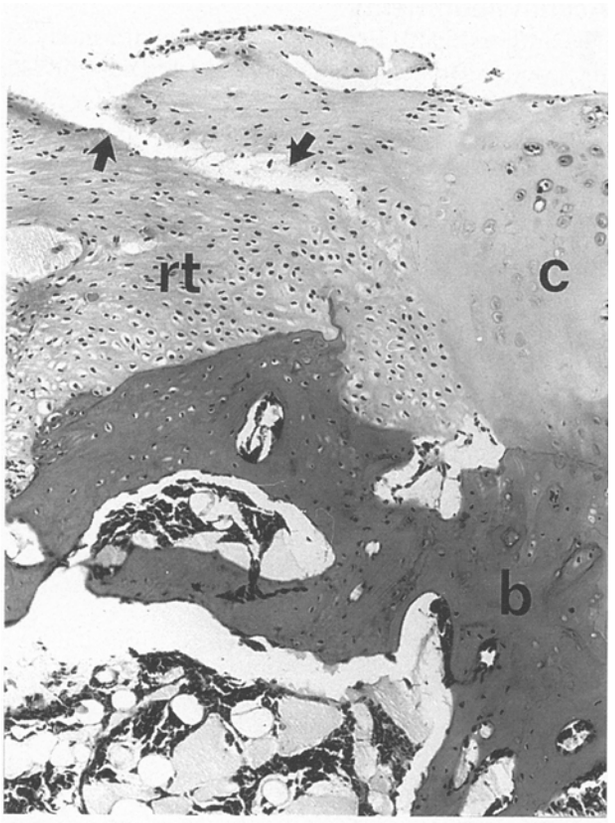

Figure 7 Junction between repair tissue and normal articular cartilage in the control group, with no biomaterial implanted into the defects. The defect has been filled with fibrous tissue, poorly integrated with the adjacent articular cartilage. The surface is irregular and deep fissures can also be seen. (rt = repair tissue, c = normal articular cartilage, b = subchondral bone, the arrows show a fissure). [Haematoxylin-Eosin staining, x 20]

intra-articular implantation [5]. In this study, synovial samples were also studied and confirmed that the polymer is biocompatible. Mild foreign body reaction to polymer particles was noted in both polymer groups, with no significant difference between the new polymer and the conventional bone cement.

In the present experimental model, the polymers were implanted well into the subchondral bone, below the level of the surrounding articular cartilage. Subchondral marrow cells can potentially differentiate to chondrocytes [9, 10] and thus, exposure of subchondral bone is a potential biological advantage. PEMA/THFMA has been found to be hydrophylic *in vitro* [11] and it appears to demonstrate low shrinkage characteristics during polymerization [12]. Providing that these properties are maintained *in vivo*, the material can provide a mechanically stable system, able to distribute evenly the joint load and also able to adsorb tissue fluids, including growth factors [13–15]. Compared to PEMA/THFMA, conventional bone cement lacks hydrophylicity and in addition, it shrinks approximately 20% by volume during polymerisation [12].

It has been suggested [6], that PEMA/THFMA increases proteoglycan synthesis by the chondrocytes, thus increasing resistance to compressive mechanical loading, applied to the repairing defect. It has also been shown [16] that the capability of the articular cartilage to repair is related to stress placed upon it and that excessive loading can be harmful. We therefore postulate that the larger amount of proteoglycans, secreted by chondrocytes in the PEMA/THFMA group, provides better mechanical shelter to them and also to undifferentiated mesenchymal cells that migrate in the repairing defect and differentiate to new chondrocytes.

It is evident that, besides mechanical factors, the overall repair process is also influenced by biological factors. The exothermic reaction, generated by both polymer systems during polymerization, is a critical biological factor. Compared to conventional bone cement, PEMA/THFMA generates significantly less energy while polymerising *in situ* [17, 18], thus preventing/eliminating cell damage and death.

5. Conclusions

We conclude that the new, hydrophylic polymer PEMA/THFMA, when implanted into the subchondral bone, enhances the biological repair process in large articular cartilage defects. Compared to control specimens, in which no biomaterial was implanted, conventional bone cement did not improve significantly the quality of the repair. PEMA/THFMA is biocompatible and the method of its implantation simple and easily reproducible, thus promising for clinical application.

Acknowledgments

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