# Biomechanical and histological evaluation of hydrogel implants in articular cartilage

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## Abstract

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We evaluated the mechanical behavior of the repaired surfaces of defective articular cartilage in the intercondylar region of the rat femur after a hydrogel graft implant. The results were compared to those for the adjacent normal articular cartilage and for control surfaces where the defects remained empty. Hydrogel synthesized by blending poly(2-hydroxyethyl methacrylate) and poly(methyl methacrylate-co-acrylic acid) was implanted in male Wistar rats. The animals were divided into five groups with postoperative follow-up periods of 3, 5, 8, 12 and 16 weeks. Indentation tests were performed on the neoformed surfaces in the knee joint (with or without a hydrogel implant) and on adjacent articular cartilage in order to assess the mechanical properties of the newly formed surface. Kruskal-Wallis analysis indicated that the mechanical behavior of the neoformed surfaces was significantly different from that of normal cartilage. Histological analysis of the repaired defects showed that the hydrogel implant filled the defect with no signs of inflammation as it was well anchored to the surrounding tissues, resulting in a newly formed articular surface. In the case of empty control defects, osseous tissue grew inside the defects and fibrous tissue formed on the articular surface of the defects. The repaired surface of the hydrogel implant was more compliant than normal articular cartilage throughout the 16 weeks following the operation, whereas the fibrous tissue that formed postoperatively over the empty defect was stiffer than normal articular cartilage after 5 weeks. This stiffness started to decrease 16 weeks after the operation, probably due to tissue degeneration. Thus, from the biomechanical and histological point of view, the hydrogel implant improved the articular surface repair.

## Introduction

Different synthetic and biological materials have been studied for use in the repair of osteochondral defects (1-6), but to date no method has been reliable in restoring a functional articular surface. Among the different synthetic materials that have been studied, hydrogels are special (7,8). Hydrogels are obtained from hydrophilic polymers with covalent bonding between macromolecules, resulting in a network that is able to absorb large amounts of water (9). In addition to being compliant, when implanted hydrogels allow the normal flow of body fluids responsible for cell maintenance. Since they are

physically similar to soft tissues, especially articular cartilage, these gels have appropriate mechanical properties and may be used as a graft for the repair of articular cartilage defects. Their surface and functional properties should provide the necessary mechanical support for the joint (10).

The mechanical properties of articular cartilage as well as its functional performance are related to the tissue structure, which consists of a fluid phase (water and electrolytes) and a solid phase. The solid phase is a complex network of collagen with proteoglycan aggregates, forming an incompressible matrix permeable to synovial fluid (11). The indentation test is the most commonly used method for evaluating the mechanical behavior of articular cartilage (12-15).

In this study we evaluated the mechanical performance of the tissue formed in defects produced on the articular surface of the intercondylar region of rat femurs in the absence and presence of a hydrogel implant. We considered the intercondylar region the most appropriate for this study because of the large area available to perform the defects. This region is not a weight-bearing area, but is submitted to friction and wear due to the sliding of the patella during the movements.

## Material and Methods

Hydrogel was obtained by thermal polymerization (85°C/4 h) of a mixture of 96% (w/w) 2-hydroxyethyl methacrylate (Aldrich Chemicals Co., Milwaukee, WI, USA), 5.0% (w/w) poly(methyl methacrylate-co-acrylic acid) (Metacril, Camaçarí, BA, Brazil), 1% (w/w) trimethyleneglycol trimethacrylate (Retilox, São Paulo, SP, Brazil) and 0.5% (w/w) benzoyl peroxide (Laport Chemicals, Santo André, SP, Brazil). Glucose crystals were added to this solution in order to obtain a porous structure. The resultant gel consisted of two layers: a porous layer 1.5 mm

thick (with a diameter of less than 45 μm) and a dense layer 0.5 mm thick. After synthesis the hydrogel was washed in distilled/deionized water for glucose removal and immersed in saline solution at pH 7.0 for pH equilibrium. Before implantation it was sterilized in an autoclave for 30 min at 120°C (16,17).

### Animal model

Male Wistar rats (~200 g) were anesthetized by ether inhalation. The knee joint was accessed by a medial parapatellar incision and a cylindrical hole 2 mm deep x 2.5 mm in diameter was made in the intercondylar region of the femur. The orifice was washed with saline solution, and a sample of hydrogel of the same size as the orifice was implanted in the left knee while the control orifice in the right knee remained empty. The animals were divided into five groups of five animals per group and were observed 3, 5, 8, 12 and 16 weeks after the operation.

### Mechanical analysis

The indentation test was performed on the repaired and normal articular surfaces of rat femurs. The advantage of this approach is that the mechanical behavior of the natural cartilage can be evaluated without removing it from the bone. The load was applied to the cartilage using a spherical stainless steel (310 L) tip (diameter 1.0 mm). A universal material test system (MTS model 810) was used for the test. The specimen was immersed in 0.15 M NaCl solution at 37°C and was fixed in a position appropriate for surface indentation (perpendicular to the tip). After the tip touched the surface lightly, the surface was deformed at a rate of 0.5 mm/min, and the load needed for this deformation was recorded. After attaining a deformation of 0.3 mm, the deformation was maintained constant for a period of 120 s and the stress relaxation of the tissue was recorded. Then,

the load versus time curves were recorded throughout the test.

Indentation tests were performed in the control and implanted defect areas. The normal articular cartilage was evaluated in at least three places around the area of the defect. Five animals were evaluated per group and the average values from the five curves were used to analyze the results throughout the 16 weeks following the operation.

The values of the load measured to obtain a 0.16-mm indentation depth were used to calculate the elastic modulus of the indented areas. A small deformation of the surface was chosen for use in the calculations of the elastic modulus.

### Histological analysis

After the mechanical test, the joints were submitted to histological analysis. The specimens were fixed in 10% paraformaldehyde solution for 48 h, decalcified with 5.0% (v/v) nitric acid, washed and dehydrated with a gradual series of ethanol and xylene solutions. After this, they were embedded in wax blocks and sagittal sections, including the adjacent repair tissue, were cut using a rotary microtome (LEICA RM 2155) and stained with Masson's trichrome. The stained sections were observed and photomicrographed using an Olympus BX60 light microscope equipped with an automatic exposure photomicrograph system PM 10AK3.

### Statistical analysis

The Kruskal-Wallis test ($\alpha = 0.01$) was used to compare the results of the indentation tests for different situations (implant, control and normal cartilage). Linear regression was used to evaluate the influence of postoperative time on the stiffness of different repair tissues.

## Results

Figure 1 shows typical curves for the load pattern during the indentation test (load x time) for normal (A), control (B) and implanted (C) articular surfaces. During the first 36 s, the indentation phase, the load was increased until a depth of 0.3 mm had been reached. The load necessary to reach this deformation was dependent on surface stiffness. In the second phase of the test, the load was decreased slightly as a consequence of tissue stress relaxation.

The pattern of the indentation curves obtained for normal cartilage remained unchanged during the 16 weeks of observation. Although the loads needed for deformation of the tissue formed over hydrogel increased significantly during the postoperative period, they did not reach the level of those for normal cartilage even within 16 weeks. In contrast, the loads needed for the tissue formed on the control defects were significantly higher than those for normal cartilage, but decreased over the 16-week period.

Statistical analysis by the Kruskal-Wallis test ($\alpha = 0.01$) showed that the average curves resulting from the indentation tests on the normal, control and implanted surfaces were significantly different for all postoperative periods.

The results of the mechanical evaluation of normal and repaired articular surfaces can be observed in Figure 2. The figure shows the elastic modulus values obtained with the
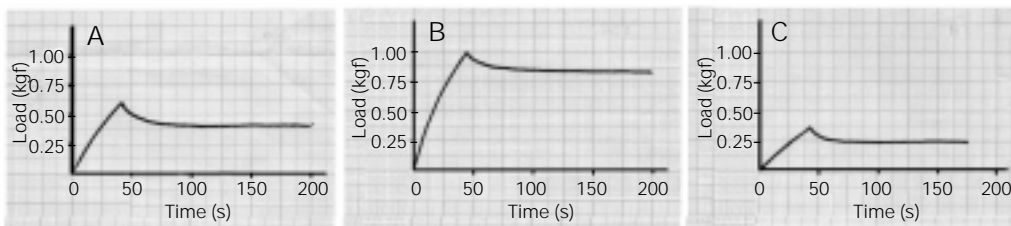
Figure 1 - Typical load versus time curves obtained as a result of the indentation tests. A, Normal articular cartilage surfaces; B, control repaired surfaces, and C, surfaces repaired with the hydrogel implant.

indentation test of normal articular surfaces, control repaired surfaces and surfaces repaired with the hydrogel for different postoperative periods. Variations in the elastic modulus of normal articular surfaces were observed for the different groups, probably due to experimental errors caused by the difficulty in finding a plane surface in the small knee joint of the rat. In the case of repaired surfaces there was a flat surface in the implanted area of the articular surface. For the surfaces repaired with the hydrogel implant the elastic modulus values were lower than those for the normal surfaces in all groups. Initially the elastic modulus of the surface was similar to that of the hydrogel

before implantation but increased with time following the operation. The elastic modulus of control repaired surfaces increased to values similar to those for articular cartilage after 5 weeks, showed higher values after 5 weeks but showed a decrease after 16 weeks.

Linear regression of the elastic modulus values obtained for the different postoperative periods yielded a correlation coefficient of 0.77 for repair of the implanted surface and confirmed the tendency towards an increase in the stiffness of the neoformed surface during the period after implantation. For control repaired surfaces, the coefficient was 0.429, since the elastic modulus value decreased for 16 weeks after the operation. For normal cartilage, the correlation coefficient value was -0.19.

The histological aspects of the repaired defects in the control and implanted groups 8 and 16 weeks after operation are shown in Figures 3 and 4, respectively. In the control repair situation, the defect was filled with osseous tissue with a very thin layer of fibrous tissue on its surface (Figure 3). This fibrous tissue was not well integrated with the articular cartilage around the defect. The control defects were repaired by tissue growing from the margins towards the center of the defect, resulting in irregular surfaces even after 16 weeks (Figure 3B).

Figure 4 shows that in implanted surfaces the hydrogel graft filled the defect with no signs of significant inflammation, and was well anchored to the implant site due to the growth of surrounding tissue into the hydrogel pores. The hydrogel implant repaired the defects, resulting in regular artificial articular surfaces (Figure 4A and B). A detailed evaluation of the biological performance of this hydrogel in the repair of articular cartilage defects was described in a previous report (18).

## Discussion

Many studies involving indentation tests

Figure 2 - Average values of the elastic modulus obtained from experimental measurements of load needed to make a 0.16-mm indentation depth. Data are shown for normal articular cartilage surfaces, control repaired surfaces and surfaces repaired with the hydrogel implant for different postoperative periods.
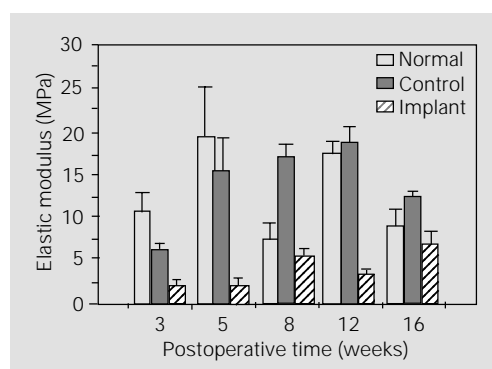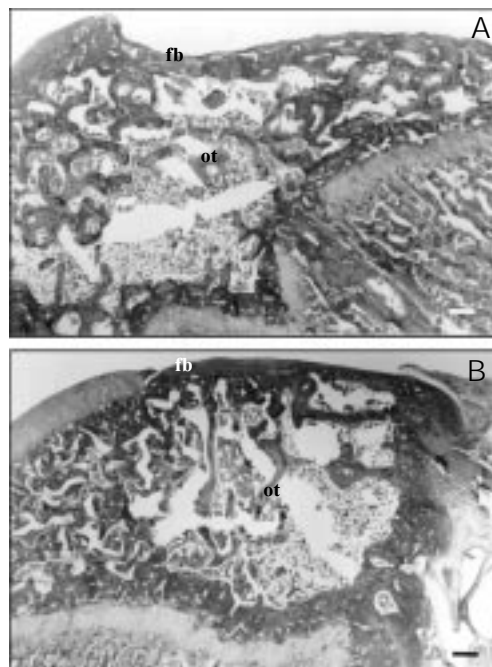


Figure 3 - Section of a repaired control defect stained by Masson's trichrome (A) 8 weeks after operation and (B) 16 weeks after operation. Note the newly formed osseous tissue (ot) filling the defect and the fibrous tissue (fb) on the superficial zone. Magnification bar = 200 μm.

have been recently conducted to evaluate the mechanical behavior of articular cartilage (12-15,19). In the present study we chose to use the model used by Kempson et al. (12) and the results obtained showed that the mechanical performance of the repaired surfaces of control and hydrogel graft implants differed from that of hyaline cartilage.

The neoformed tissue in the control group showed an increase in stiffness during 12 weeks following the operation and a decrease for the 16th week. It is difficult to compare the stiffness of the control and implant repaired surfaces with the stiffness of normal articular cartilage surface since the latter showed wide variation in the elastic modulus values during the experiment (7.5-20.0 MPa). Nevertheless, if we consider an average value for the elastic modulus of normal cartilage of about 13.0 MPa with time, we can see that after the 5th week following the operation, the stiffness of the repaired surfaces was greater in the control group compared to normal articular cartilage. The increased stiffness of the neoformed tissue in the control group can be justified by the occurrence of bone neoformation during the filling up of the defect and the formation of fibrous tissue on the surface (Figure 3). This tissue does not have the same compliance as normal cartilage and seems to start to degenerate after 16 weeks. According to some investigators (10,20), in the case of articular resurfacing by drilling the osteochondral surface, repair tissue is formed as a result of differentiation of mesenchymal cells into osteoblasts, chondroblasts or fibroblasts and does not show good integration with neighboring cartilage. This lack of integration between tissues causes micromovements that contribute to degeneration of the neoformed surface.

Although the surface repaired by the hydrogel implant was not as stiff as normal cartilage, it seemed to show a more appropriate mechanical and physiological performance. The lower degree of stiffness of the

surface will not cause damage to the opposite articular surface. The results of linear regression suggest that if the postoperative period were longer, the neoformed surface might behave in the same manner as articular cartilage.

The hydrogel is able to work as a damping material. This fact has to be considered when interpreting these data, since this effect is probably important in determining the durability of a restored surface by protecting it from the wear and tear that normally occurs between two articular surfaces.

The growth of tissue into the hydrogel pores may have contributed to stabilizing the implant inside the defect, resulting in the improvement in surface stiffness throughout the postoperative period. In this study we did not observe tissue growth on the top of the hydrogel surface. It seems that if tissue was growing on the top surface during the first weeks after the operation, it did not adhere well to the hydrogel surface and consequently was removed due to the sliding of the patella against the repair site during the joint movement. In spite of this, the hydrogel remained inside the implant site working as an artifi-
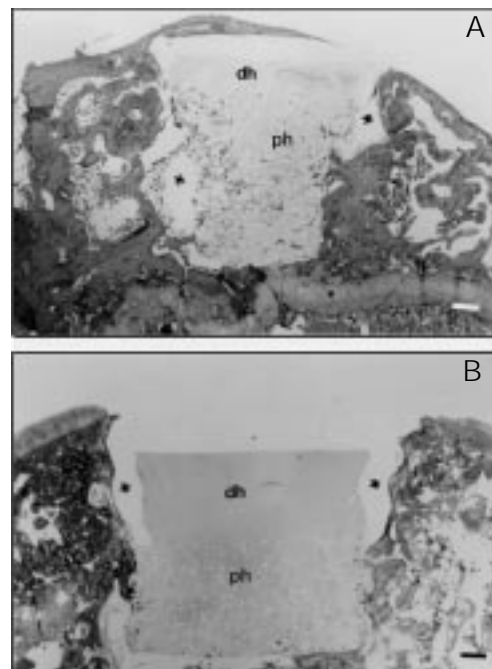


Figure 4 - Section of the defect repaired with the hydrogel implant stained by Masson's trichrome (A) 8 weeks after operation and (B) 16 weeks after operation. The dense hydrogel (dh) top surface and the porous hydrogel (ph) substrate filling the defect can be seen. The implant seems to be well anchored to the surrounding tissues. * = Experimental artifact caused by hydrogel shrinkage during the dehydration of the section. Magnification bar = 200 μm.

cial articular surface.

No similar studies with hydrogel implants have been reported in the literature. In 1990, Corkhill et al. (7) published a study on the potential of synthetic hydrogels for the repair of articular cartilage, but to date no results of *in vivo* tests have been published. Reissis et al. (8) and Downes et al. (21) have studied the use of a poly(ethyl methacrylate)/tetrahydrofurfuryl methacrylate hydrogel for the regeneration of articular cartilage,

but they have not evaluated the mechanical behavior of the repaired surfaces.

Although the results obtained here indicate that hydrogel is beneficial to the repair of osteochondral defects, they are not yet conclusive. Further studies on large animal species and with longer follow-up periods are currently underway in order to improve our understanding of the action of this polymer in articular cartilage repair.

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