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Long-term survival of regenerated cartilage on a large joint surface

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Abstract—The one-year survival of regenerated cartilage on a large articular surface is presented using the McDowell *in vivo model.*[1] The model provides a mechanically shielded environment in which regenerated cartilage can be protected from intra-articular stresses while normal joint motion is maintained. New tissue was allowed to grow from bleeding subchondral bone for 12 weeks at which time the original mechanical environment was reintroduced. Our study showed that neo-cartilage would grow to cover the entire joint surface of a patella and could survive for one year. Histologic observations indicated a maturing hyaline-like tissue. Biomechanical analyses showed that the regenerated cartilage became stiffer and less permeable within the time of this study. Biochemical evaluations demonstrated stable properties out to the longest time point. Control specimens, which were not shielded from stress, showed insignificant amounts of new tissue growing on the patellar surfaces.

Key words: *articular cartilage, biomechanics, regeneration, stress-shielding.*

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[1]U.S. Patent #5,108,441

INTRODUCTION

Durable cartilage repair continues to be a vexing issue faced by clinicians and scientists alike. The majority of recent efforts have concentrated on developing a successful biological resurfacing technique that would create a living tissue on an articular surface that can maintain its homeostasis, i.e., respond/adapt to the stresses required through cellular control of its biochemical components and thus its biomechanical function.

Reparative techniques that employ grafting chondrogenic tissue (either as autografts or allografts) into full-thickness defects have been studied (1–4). The purpose of grafting these tissues into the defects is to introduce a cell source to regenerate the tissue. Improvements in the quality of the regenerated cartilage were found over other techniques such as puncture arthroplasty or chondral abrasion, but only in the short-term. Tissue engineering approaches have also been under intense investigation. Cells such as chondrocytes are incorporated into a scaffolding matrix such as fibrin glue or collagen prior to transplantation into a defect (5–8). Variable results have been produced. Some small defects do well in the short term. Bonding to bone and adjacent normal cartilage is a problem. Recent attention has focused on autologous

chondrocyte transplantation (9). *In vivo* studies however have not yet been able to match the reported clinical success of the technique (10). In this canine study, no detectable difference could be discerned between defects repaired with or without autologous chondrocyte transplantation.

Currently, no method has yet been proven satisfactory or predictable for repairing damaged articular cartilage that encompasses a large portion of the joint surface and is durable in the long term (11). One of the major reasons new cartilage will not grow in a clinically relevant amount on a large joint surface is that mechanical factors such as abrasion and compression destroy the immature new tissue (12). This study hypothesizes that regenerated cartilage will grow and can be maintained in the long-term on a large articular surface in a moving joint if the new tissue is shielded from abrasive and compressive forces during the early stage of regeneration. We believe that keeping the new tissue in an environment that is close to normal, except for temporarily relieving mechanical stress, will create a stable new tissue. The model was designed to preserve joint motion. This study presents the multidisciplinary evaluation of cartilage grown in the model out to 1 year.

MATERIALS AND METHODS

Experimental Model

The canine patellofemoral joint was selected as the site for evaluation of the *in vivo* model. Fifteen skeletally mature mongrel dogs, approximately 25 kg, were used guided by a protocol reviewed and approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. The animals underwent bilateral surgeries in which medial parapatellar incisions were made into the knee joints. At the first operation the patellae were everted, and the articular cartilage of the patellae was removed down to bleeding subchondral bone with a high speed burr cooled by continuous irrigation with sterile saline, according to the method described previously (13). The patellar surface was protected from contact with the patellofemoral groove by insertion of joint spacers which permitted full joint motion but separated the two articulating surfaces for 12 weeks, long enough for cartilage to regenerate on the denuded surface (14). Two 3.5 mm diameter holes were drilled into the proximal and distal poles of the patellae, and two small threaded, dome-shaped, high-density polyethelene spacers were inserted. The dome-shaped surface of the spacers protruded 2–3 mm into the patellofemoral joint and lifted the surface of the patellae off the patellofemoral groove, giving a stress-shielded area over the patellae on which regenerated cartilage could grow. Incisions were closed in standard fashion. Animals were permitted free activity in a large pen and taken outdoors 5 days per week to walk, run, and jump.

The animals then underwent a second surgery at 12 weeks to remove the spacers and to introduce the regenerated cartilage to the stress environment. Five animals each were euthanized at 24, 32, and 40 weeks after this second surgery. Two additional animals were evaluated at 52 weeks as controls. Control specimens were not allocated to all time points because almost no new tissue grew on the unshielded control surface up to 18 weeks (13). It would have been wasteful to look at growth at all long-term time points. Thus, the only control in this project was 52 weeks. For this time point, one knee of one animal and both knees of a second animal served as control.

Evaluations of the regenerated cartilage included scoring of the gross appearance and histological qualities via a modified grading scheme (15,16) (**Table 1**) and quantification of the biomechanical properties and biochemical characteristics. The patellae were split in half with a sharp osteotome. The proximal half of the patella was quantified biomechanically and biochemically while the distal portion was evaluated histologically. Based on a canine study (17), the majority of the surface area of the patella is subjected to stresses at different angles of flexion. Thus, all sites of evaluation would constitute load-bearing regions of the patellar surface. Similar evaluations were performed on the patellofemoral grooves to assess the extent of any changes to this surface. The grooves were equally divided into three transverse sections for biomechanical, histological, and biochemical analyses. Eleven normal patellae were collected to establish baseline values for comparison with the neocartilage grown on the bone surface of the experimental patellae before and after the neocartilage was subjected to compression and shear stresses within a moving joint.

Histologic Analysis

Specimens for morphological evaluation were processed using the stains Haematoxylin and Eosin (H&E) and Safranin-O. The tissue was evaluated grossly for shape and contour and histologically for staining and

Table 1.
Objective grading criteria for histological and gross methods for analyzing cartilage in the repair model

Patella Evaluation	Grade	Groove Evaluation	Grade
I. Extent of coverage		**I. Gross appearance**	
>75% surface area	4	Normal/no wear of cartilage	4
50-75% surface area	3	Slight wear/minimal loss of normal color	3
25-50% surface area	2	Moderate wear/visible areas of subchrondral bone	2
<25% surface area	1	Significant wear/multiple denuded areas	1
No new cartilage	0	Severe wear/small areas of remaining cartilage	0
II. Cartilage color		**II. Hypocellularity**	
Normal	4	None	3
Yellow/slight loss of translucency	2	Slight	2
Brown/moderate loss of translucency	0	Moderate	1
		Severe	0
III. Cartilage surface		**III. Chrondrocyte clustering**	
Smooth	3	None	2
Irregular	2	<25% of cells	1
Shallow clefts	1	25-100% of cells	0
Clefts to tidemark	0		
IV. Neocartilage thickness		**IV. Surface integrity**	
100% of normal	2	Normal	4
50-100% of normal	1	Surface irregularities	3
<50% of normal	0	<50% loss of cartilage thickness	2
V. Safranin-O staining		>50% of cartilage thickness	1
Normal	3	Denuded cartilage to tidemark	0
Slight reduction	2		
Moderate reduction	1		
Severe reduction	0		
VI. Cell Morphology			
Hyaline-like cartilage	4		
Mostly hyaline-like cartilage	3		
Hyaline and fibrocartilage	2		
Fibrocartilage	1		
Nonchondrocytic cells	0		

uniformity of the cartilage. Cellular details included shape and distribution of the cells within the matrix. Each specimen of cartilage and the underlying bone was fixed in buffered formalin for 2–3 days, decalcified in a sodium citrate/formic acid mixture and processed for paraffin embedding. Serial sections were cut on a rotary microtome.

For H&E evaluation, sections were stained in haematoxylin for 2–5 minutes, washed well in running alkaline tap water until nuclei turned blue, counterstained with 1 percent aqueous eosin for one minute, dehydrated in alcohol, cleared in xylene and mounted in a synthetic resin medium. Nuclei appear blue to blue/black while the matrix is pink or light blue.

For Safranin-O evaluation, sections were stained with Weigert's haematoxylin for 10 minutes, washed well in running tap water until the nuclei turned blue, treated with 0.1 percent fast green solution, rinsed in 1 percent acetic acid, counterstained with 0.08 percent aqueous Safranin-O, dehydrated, cleared and mounted. Safranin-O is an orthochromatic dye which selectively stains glycosaminoglycans (GAGs).

Quantification of Biomechanical Characteristics

The mechanical properties of the repair cartilage on each patella and the cartilage on the patellofemoral groove were quantified by experimental testing in the indentation configuration and were based on a biphasic compositional view of the tissue. The properties determined were aggregate modulus (a measure of the stiffness of the tissue) and apparent permeability (indicates the ease with which fluid flows within the tissue) via a nonlinear regression algorithm (18).

Specimens for biomechanical analysis were wrapped in saline-soaked gauze, sealed in airtight plastic bags, and frozen at −85 ˚C. Then, they were defrosted at room temperature in preparation for testing. Specimens were tested *in situ* (intact with underlying bone) by using a custom-designed indentation apparatus. This device measured the instantaneous and time-dependent creep behavior of the cartilage under the application of a constant load. It consisted of a loading shaft with a porous (10μ) indenter tip through which the load is applied to the cartilage surface. Air bearings are used to eliminate the friction along, and the rotation of, the loading shaft. The test specimen was mounted in holding clamps onto the device and submerged at room temperature in 0.9 percent saline solution for 10 minutes. The chosen testing site was oriented in three-dimensional space via a ball hinge so that the 1.5 mm diameter, cylindrically shaped indenter was perpendicular to the articular surface. After equilibration for 15 minutes under a small preload, a weight was applied to the cartilage, and the instantaneous and creep deformation measured over a period of 45 minutes or until equilibrium was reached. Equilibrium was set as a small value of the slope of the creep curve (10^{-06} mm/s). Paired sets of data were collected from each indentation test—time of creep and surface compression—for use in property determination. A baseline of biomechanical properties for normal patellar cartilage from patellae harvested in other non-surgical models was used as external normals to which experimental values were compared.

Quantification of Cartilage Composition

Specimens for biochemical analysis were wrapped in saline soaked gauze, sealed in airtight plastic bags, frozen at −85 ˚C, and shipped overnight to Columbia University for evaluation. Cartilage samples from the experimental patellae and patellofemoral grooves were analyzed to determine water, proteoglycan and collagen contents as described previously (19). To determine the water content, the samples were weighed before and after freeze-drying for 48 hours. The tissue was then digested with papain (1.25 mg/100 mg tissue) for 16 hours at 60 ˚C, and aliquots of this digest were taken for separate analyses to determine hydroxyproline (as a measure of collagen) and sulfated glycosaminoglycan (S-GAG, a measure of proteoglycan).

To determine hydroxyproline content, the papain digest was hydrolyzed with HCl at 107 ˚C overnight, dried, and then analyzed by a colorimetric procedure (20). The S-GAG content was determined by using the dye 1,9-dimethylmethylene blue (21), adjusted for use with a microtiter plate (19).

Statistical Analysis

The data obtained from the biomechanical and biochemical analyses were assessed for statistical differences using a one-way analysis of variance (ANOVA) for the effect of postoperative time. Post-hoc multiple comparisons were performed with Dunn's method. Significance was set at $p=0.05$.

RESULTS

Gross and Histologic Evaluation

None of the spacing devices was broken or loose. There was evidence of minimal wear of the dome-shaped surface that reached a maximum of 0.5mm in less than 10 percent of the devices. All animals were walking by the second day after surgery, and all knee joints obtained a full range of motion by 6 weeks after each operation.

Gross specimens and histologic tissue sections were scored according to the scheme presented in **Table 1** (modified from 15,16). Significant new cartilage growth was seen on all experimental patellae harvested (**Table 2**, **Figure 1**). After the spacers were removed at 12 weeks, the new tissue was placed in contact with the femoral joint surface and therefore exposed to shear and compressive stress. We observed that the new tissue survived and maintained its integrity and thickness for the 40 weeks of this study. Data from 12 week specimens (13) is included for comparison purposes—these specimens were shielded from stress for 12 weeks and then animals were sacrificed.

Gross inspection of the 12+40 week specimens showed that 75–100 percent of the patellar surface area was covered with new tissue. Using color of the new tissue as a relative guide, we observed no degradation in

Table 2.
Cartilage characteristics (mean [std. dev]) from long-term specimens

	Normal	12 weeks	12+24 weeks	12+32 weeks	12+40 weeks	Control 52 weeks
GROSS and HISTOLOGY						
Patellar cartilage						
I. Extent of coverage	4	3.4(0.8)	3.6(0.5)	3.4(0.54)	3.7(0.5)	2.0(1.0)
II. Cartilage color	4	2.9(1.1)	2.6(0.5)	2.2(0.8)	2.9(0.5)	1.2(1.0)
III. Cartilage surface	3	1.1(0.4)	1.0(0.5)	0.8(0.8)	1.0(0.8)	0.3(0.6)
IV. Neocartilage thickness	2	1.6(0.5)	0.8(0.8)	1.4(0.7)	1.0(0.6)	0.0(0.0)
V. Safranin-O staining	3	1.2(0.4)	1.1(1.0)	1.0(0.5)	0.8(0.6)	1.3(1.2)
VI. Cell morphology	4	1.3(0.5)	2.1(0.3)	1.9(0.3)	2.0(0.8)	0.7(1.2)
Groove cartilage						
I. Gross appearance	4	2.0(0.6)	2.2(0.3)	2.33(0.66)	2.32(0.84)	1.00(0.00)
II. Hypocellularity	3	1.4(0.8)	2.4(0.7)	2.67(0.50)	2.45(0.69)	0.33(0.58)
III. Chondrocyte clustering	2	0.1(0.4)	0.4(0.5)	0.89(0.60)	0.55(0.52)	0.00(0.00)
IV. Surface integrity	4	1.1(0.7)	2.2(1.2)	2.56(0.88)	1.90(0.94)	0.33(0.58)
BIOMECHANICS						
Patella: Thickness (mm)	0.67(0.28)	1.65(0.33)	*0.95(0.29)	*1.00(0.18)	*0.97(0.21)	n/a
BIOCHEMISTRY						
Groove: Hydration (%)	66.5(4.7)	*75.3(2.8)	*75.0(1.0)	75.8(2.1)	*75.0(1.2)	*74.5(1.1)
Hydrosyproline content	54.4(20.0)	*56.5(12.6)	*65.9(10.0)	*67.4(7.5)	*63.2(8.0)	84.2(19.5)
Proteoglycan content	138.2(18.0)	*142.8(23.8)	104.4(24.7)	*122.2(24.2)	95.8(20.1)	105.9(22.8)

Biochemical measures in mg proteoglycan or hydroxyproline/gram of dry tissue. n/a indicates not enough tissue regenerated to perform analyses. 12+"X" indicates 12 weeks of stress relief followed by "X" weeks after spacer removal.) *≡not satistically different from normal (p>0.05).

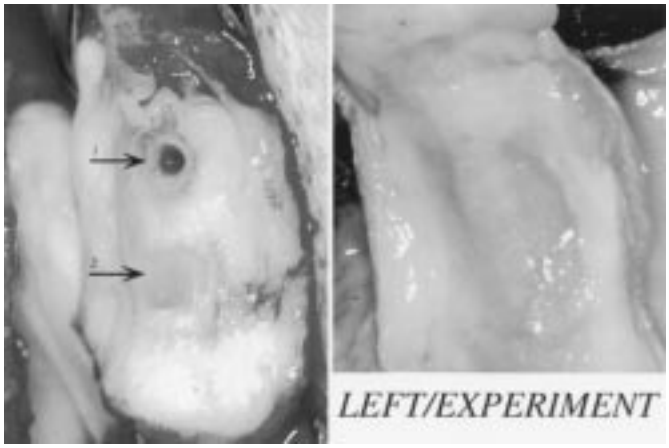

Figure 1.
Gross photographs of patellofemoral joint during 2nd surgery at 12 weeks to remove spacers (left image) and 40 weeks after 2nd surgery (right image). Note the abundance of new cartilage coverage on the patella at 12 weeks and the sustained coverage after 40 weeks of joint stress. The left image shows one spacer not yet removed (arrow 2) and the margin left after spacer removal (arrow 1). Note also that the holes left by the spacers after removal at 12 weeks were completely filled in and incorporated into the surrounding tissue in this 12+40 week specimen (right image).

the quality of the repair tissue between 12+24 and the longer-term 12+40 week specimens. Clefts in the surface of the new tissue were observable grossly and microscopically in all specimens (**Table 2**). Histologic examination showed that some of the clefts extended to bone.

After having been exposed to joint stresses for 40 weeks, the new tissue showed areas of hyaline cartilage and fibrocartilage. A fibrous tissue stroma predominated, but chondrocytes were frequently observed in a columnar distribution within an amorphous, ground glass stroma (**Figure 2A**). This finding correlated well with Safranin-O staining for proteoglycans, which indicated the presence of hyaline tissue (**Figure 3**). We graded Safranin-O staining, according to the previously established criteria (**Table 1**), as a "moderate reduction" compared to normal tissue. The thickness of the new tissue after 12 weeks of growth and maturation in the shielded environment varied between 3 mm and 0.6 mm, depending upon how close to the spacer the measurement was made. The thickness was greatest adjacent to the spacer. New tissue thickness diminished after it was placed into contact with the femoral articular surface (**Table 2**).

Control patellar specimens were created by removing all normal cartilage from the patella. Spacers were not inserted, and thus, there was no shielding of the exposed

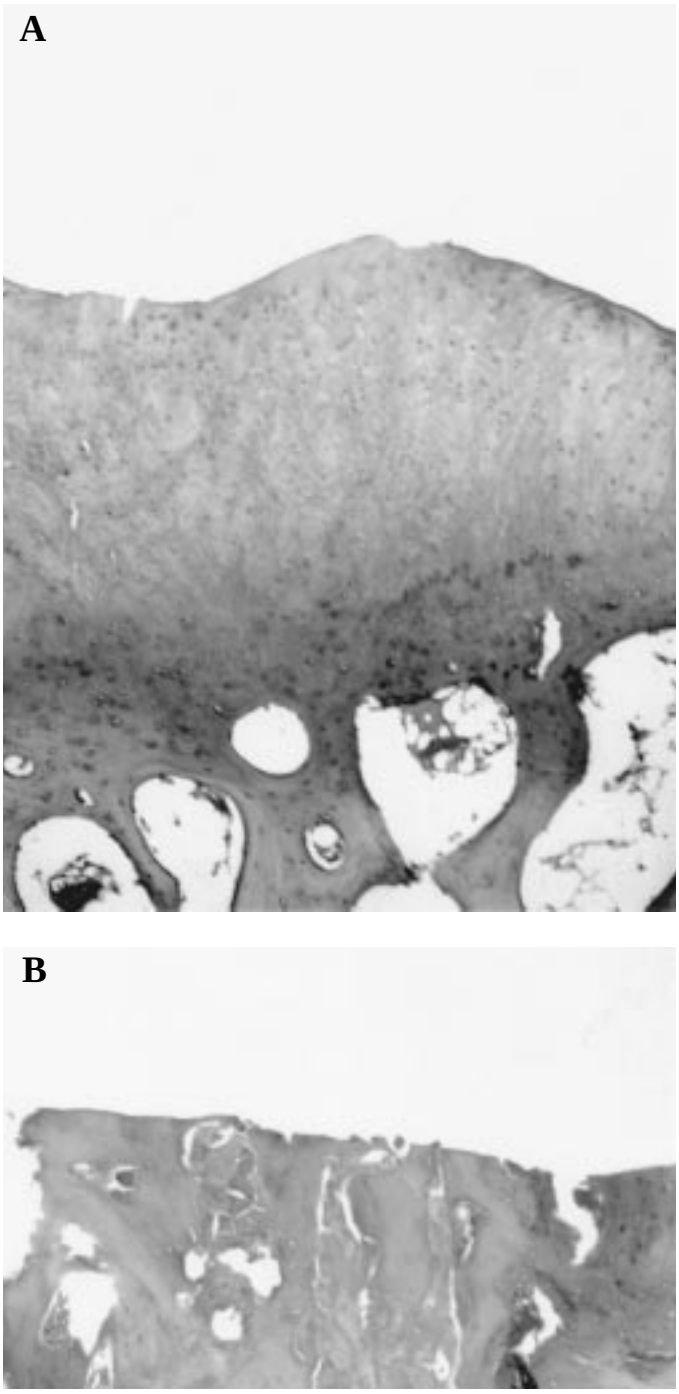

Figure 2.
Light micrograph (H&E; 48×) of: A) new tissue that grew from the subchondral bone on one canine patella. The new tissue grew in a shielded environment for 12 weeks and then the shielding devices were removed so that the new tissue was subjected to joint stress against the moving contralateral joint surface for 40 weeks. There are areas of chondrocyte cloning in columns surrounded by hyaline material. B) In contrast, exposed bone remained in control (no spacers) specimens, seen at 52 weeks.

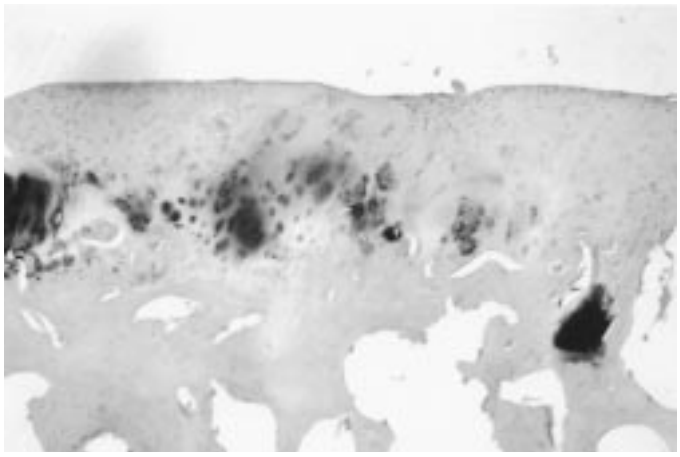

Figure 3.
Light micrograph (Safranin O; 48×) of a 12+40 week specimen, which shows staining for proteoglycans about chondrocytes within the new tissue.

bone surface. Control patellar surfaces, which were subjected to joint stresses for the entire 52 weeks, showed minimal growth of new tissue (**Table 2, Figure 2B**). The growth that did occur covered less than 10 percent of the surface of the patella and had poor qualities with wisps and tufts of tissue in small areas. The paucity of tissue made the control specimens unsuitable for biomechanical and biochemical analysis.

The femoral groove of the patellofemoral joint was placed into contact with the spacers for 12 weeks. This area was visually observed at the second operation at 12 weeks and again at sacrifice. At the periarticular margins of some specimens, there were occasional sites of new bone and fibrocartilaginous spur formation. Thinning of the articular cartilage within the femoral grooves was seen at the site where the spacers came into contact with the cartilage on the femur. The changes documented by gross evaluation of these sites at the time the spacers were removed showed no progression during the ensuing 24 to 40 weeks.

Histologic evaluation of the articular cartilage at the site where contact with the spacers occurred showed hypocellularity at the later time points. Chondrocyte clustering was common in all specimens at the site. The much larger remaining surface of normal hyaline cartilage on the femur, which never came into contact with the spacing devices, retained its normal appearance upon gross and histologic assessments. While the spacers caused some damage to the hyaline cartilage in the femoral groove, the altered surface characteristics did not appear to shear off the neocartilage on the patellae, as the

neocartilage covered the majority of the patellar surface in long-term specimens.

Biomechanical Quantification

Biomechanical analysis was performed on the cartilage in this model and on normal tissue. A site near the center of the patella was selected for testing (i.e., in between the two spacers on the experimental patellae). Good agreement was found between the theoretical description of indentation behavior and experimental data (**Figure 4A**). Control patellae could not be evaluated because of the minimal new tissue growth and quality noted previously.

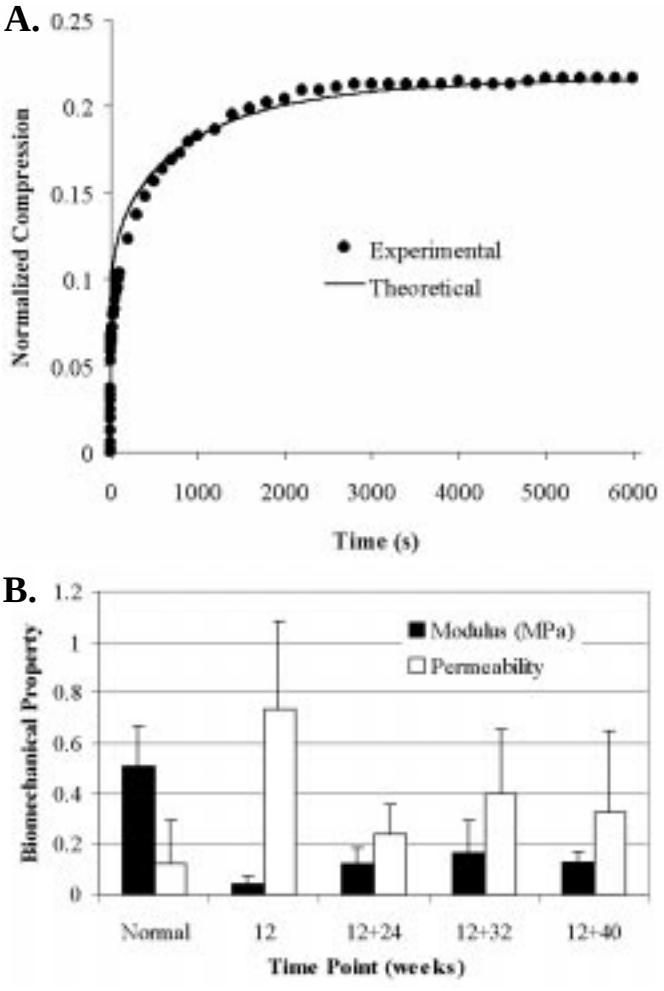

Figure 4.
A) Typical curvefit between experimental data from a 12+40 week patellar specimen and theoretical predictions to obtain biomechanical properties; B) Biomechanical qualities of the neocartilage grown on the patellar surface at the various time points, expressed as mean + standard deviation. Permeability has units of ($\times 10^{-14}$ −4/N−s). * ≡ not statistically different from normal ($p>0.05$).

Biomechanical characteristics of the new tissue indicated it to have a lower modulus than normal hyaline cartilage while the spacers were in place (12 weeks) (**Figure 4B**). The modulus demonstrated a trend of increasing with time once subjected to joint stresses, but this effect was not statistically significant ($p>0.05$). The modulus remained less than normal at all time points ($p<0.05$). Permeability of the new tissue was higher than normal while the spacers were in place ($p<0.05$) but improved with time as values at points after removal of the spacers were not significantly different from normal levels ($p>0.05$). Thickness of the new tissue was measured at the site of indentation, necessary for determination of the biomechanical properties. At this site, the new tissue was statistically thicker than normal at the 12 week time point ($p<0.05$) (**Table 2**). Thickness decreased with exposure to joint stresses such that neocartilage thickness at the later time points was not significantly different from normal ($p>0.05$). Evaluation of the patellofemoral grooves revealed that the modulus and permeability remained comparable to normal ($p>0.1$ and $p>0.5$, respectively) (data not shown). The properties for normal cartilage were similar to prior findings (22).

Biochemical Quantification

Analysis of the chemical composition of the new tissue supported the other evaluations. We found that the quality of the new tissue remained similar through to the 1-year time point (**Figure 5**). Hydration on the experimental patellae at 12 weeks was somewhat higher in comparison to normal patellae but not statistically significant ($p>0.05$). Upon exposure to the joint stress environment, the hydrated nature of the new tissue was maintained as it was still somewhat greater than normal ($p<0.05$) but not compared to that at 12 weeks ($p>0.05$). Collagen content indicated that the compositional quality of the tissue was similar to normal for all time points ($p>0.05$). Proteoglycan content was maintained up to 1 year, but still less than normal ($p<0.05$). Control patellae could not be evaluated because of the minimal growth and quality noted previously. Evaluation of the patellofemoral grooves revealed that the biochemical composition was still comparable to normal, although proteoglycan content decreased moderately at 12+24 weeks and at 1 year ($p<0.05$) (**Table 2**). Hydration was increased over normal only at the 12+32 week time point ($p<0.05$).

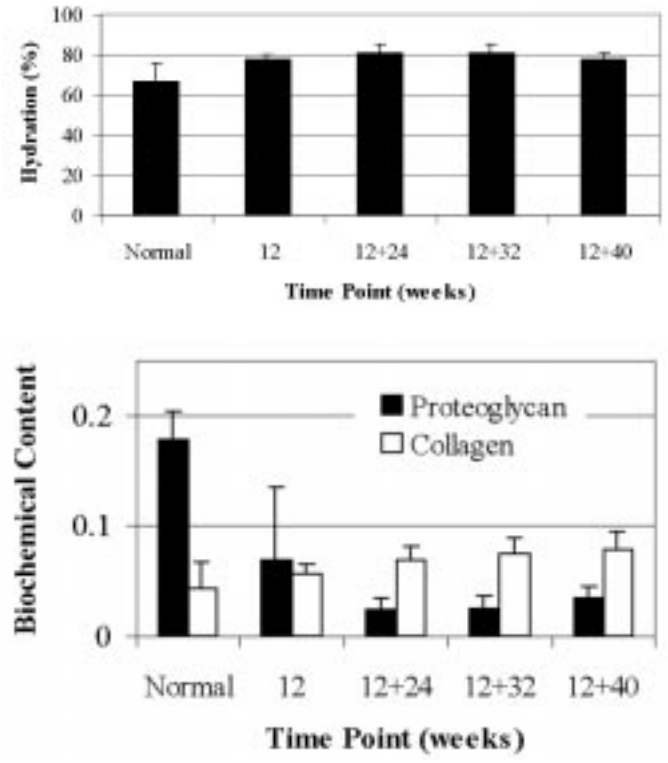

Figure 5.
Quantitative measures of the components in the patellar neocartilage, expressed as mean × standard deviation: A) Hydration; B) Proteoglycan and collagen contents, in mg/g dry weight of tissue. * ≡ (not statistically different from normal ($p>0.05$). Collagen content at all time points remained similar to normal ($p>0.05$).

DISCUSSION

Need for replacement articular cartilage continues to be unsatisfied. Interest in the subject has become stronger because of new knowledge and techniques. Enthusiasm for cartilage regeneration research exists in spite of the almost insuperable difficulties prior work has demonstrated (23).

Cartilage repair on many joint surfaces has been investigated (2,3,5,24,25). There have been some models, which use a non-load-bearing area of a joint surface or which restrict motion of a joint to protect the new tissue. Others investigate load-bearing areas without restriction of motion. Two issues of resurfacing techniques are important to consider: first, the extent of surface coverage by new tissue, and second, the ability of the new tissue to survive for years in the moving joint environment. Both of these issues have strong clinical implications. While some studies document good growth at early time points, long term results often demonstrate a degenerating tissue. The quality of the regenerated tissue in other studies is promising, but the new tissue is located in small experimentally created defects, and the tissue does not grow out onto the surface of the subchondral bone (10,16). To be clinically applicable, the size of defects covered by regenerating tissue, such as the small cylindrical or rectangular cartilage defects made in other models, must be extended to cover all of an articulating surface.

Mechanical conditions are one barrier to the creation of a useful model for neocartilage regeneration research (26). Prior models protect new tissue growth from subchondral bone stem cells or grafted materials by creating a hole that extends below the contact surface. Thus, the new tissue is protected from compression and shear stresses. The model used in this study (13) protects an entire articular surface and allows us to grow a large amount of neocartilage on the whole joint surface. The model preserves normal joint range of motion that will prevent joint stiffness and may facilitate nutrition of the neocartilage.

There have been two phases to the development of this stress-shielding model in a moving and load-bearing joint. The two phases were designed to answer two questions. First, will neocartilage tissue grow from native cells on a large joint surface and cover the majority of the joint surface if the new tissue is protected from stress during the early phase of maturation? Second, will the neocartilage grown be capable of long-term survival on the joint surface when subjected to joint stresses? The first question was answered affirmatively in the first phase (13). In this second phase, joint stresses were reintroduced in our model by removing the spacing devices. Results from evaluation of the tissue grown in the second phase answered the second question: that neocartilage grown in phase one will survive compression and shear stresses for 40 weeks. We observed that the new tissue remained firmly attached to the underlying subchondral bone. Other repair studies (12,26) observed shearing of new tissue from intact bone and cartilage. We saw a qualitative improvement in the character of the neocartilage upon restoration of joint compression and shear stresses. It is believed that the stimulus for the improvement resides in mechanotransduction in which mechanical stresses are necessary for biological materials to mature and maintain their homeostasis (27–29).

The spacing devices produced minor to moderate damage on the femoral articulating surface. Chondrocyte clustering and cloning are believed to be indicative of cartilage injury (30). However, we have to accept those

changes until a perfect spacing device can be designed that will provide stress protection during the initial growth phase of the neocartilage. We were encouraged by the lack of signs of progressive damage to the joint within the time period studied in which the neocartilage continued to improve its characteristics.

The stress-shielding approach to cartilage growth used in this model offers a unique opportunity to evaluate the potential of other techniques (e.g. native tissue from subchondral bone, periosteum, cartilage grafts, and engineered tissues) to regenerate cartilage over large joint surfaces. The model is also useful as a research tool because there is much more tissue available for study than in small drill hole defects. Finally, perhaps most importantly, once neocartilage or graft material has had time to mature in a protected environment, the protection can be removed to evaluate the ability of the neocartilage or grafted tissue to survive the compression and shear stresses in a moving joint. The model described has positive clinical implications, as replacing cartilage on a large surface is more transferable to clinical situations in many instances of injury and disease of hyaline cartilage. For example, the outcome in soft tissue arthroplasty operations done to treat arthritis or injury to the elbow and small joints of the wrist and hand is directly related to growth of neocartilage. We believe that our work will improve understanding of the process of neocartilage formation in these clinical situations and will help us achieve better, faster, and more consistent outcomes.

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