Acta Orthop. Scand. *58*, 510–515, 1987

Perichondrial autograft for articular cartilage

Shear modulus of neocartilage studied in rabbits

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Perichondrial autografts were used for the repair of large, full-thickness articular cartilage defects in the rabbit medial femoral condyle. The effects of duration of implantation and activity were studied by evaluating the neocartilage mechanically and morphologically. The complex shear moduli for the neocartilage were found to increase during the 26 weeks' observation time. Complete filling of the defect with neocartilaginous tissue was seen in a total of 24 successful experimental samples. It appeared that in the initial 6 weeks passive motion applied intermittently for 2 weeks enhanced the formation of quality neocartilage, i.e., the magnitude of the complex shear moduli was higher than those nontreated. However, these differences disappeared when longer time periods were considered.

Biological arthroplasty that aims at the regeneration of a neocartilaginous surface with the aid of either autograft, allograft, or other chondrogenic biological tissue is applicable to younger patients, as the new joint surface may have the potential to last for longer periods than artificial materials.

The growth of cartilaginous tissue from perichondrium was first noted by Tizzone (1878) and Haas (1914). The interest was renewed when Skoog et al. (1972, 1975) demonstrated that when a flap of perichondrium was raised from the ear, the vacated space rapidly filled with cartilaginous tissue. Engkvist et al. (1979) applied rib perichondrium to repair cartilage defects in rabbit femoral condyles and reported the formation of hyaline-like articular cartilage. Clinically, satisfactory results have been reported for the use of perichondrium for joint resurfacing of the wrist (Pastacaldi & Engkvist 1979); however, for the finger, results have been variable (Johansson 1979, Seradge et al. 1984).

Using rib perichondrial autograft to fill full-thickness, large articular defects in the rabbit medial femoral condyle (Coutts et al. 1984), we found production of neocartilaginous tissue that contained more type II collagen than perichondrium (Amiel et al. 1985). Little, however, is known about the biomechanical properties of the regenerated cartilage. Therefore, the objective of this study was to assess 1) the biomechanical properties of the perichondrial autograft, 2) the effects of postoperative treatment (cage activity vs. passive motion), and 3) the correlation of the mechanical findings with the reported biochemical results. A newly developed methodology was used to study the viscoelastic properties of the neocartilage. The matrix complex shear moduli under infinitesimal strain conditions were measured (Hayes & Bodine 1978, Bodine et al. 1979, Roth et al. 1982). Under these conditions, the response of the cartilage matrix was considered to be uncoupled from the interstitial fluid flow (since water cannot sustain appreciable shear force) and hence, the viscoelastic response of the cartilage matrix alone could be evaluated.

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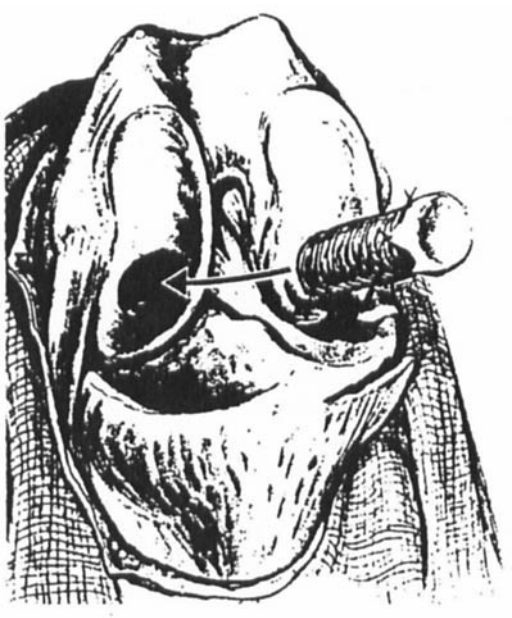

Figure 1. The experimental model. A 4-mm diameter cartilage-bone core was harvested from the medial femoral condyle, the cartilage surface was surgically removed, the denuded core was covered with perichondrial autograft previously removed from the rib and than returned to the original defect.

Materials and methods

Animal experiments. Forty-four New Zealand white rabbits weighing 3.8±0.1 kg (mean±SD) were used in this study. The animals were anesthetized with ketamine (100 mg/kg) and xylazine (8 mg/kg), and the area of surgical exposure was shaved and then prepared with betadine. On each animal, a 3.0-cm long incision was made on the left chest and a 1.5-cm portion of the first attached cartilaginous rib was obtained. The perichondrium was removed from the rib and placed in normal saline and the wound was closed (Coutts et al. 1984).

A medial parapatellar incision was then made on the left knee and an osteochondrial core from the medial femoral condyle was harvested. The core was denuded of its articular cartilage and draped with the perichondrial graft, which was sutured to the bone core. The grafted core was returned to the defect and the joint capsule and skin were closed in layers (Figure 1).

The experimental animals were divided into two groups with different postoperative treatments. One group of animals, C (cage activity), was returned to ad libitum cage activities (cage size 61 cm × 61 cm × 37 cm). The other group, P (passive motion), was placed on a machine that continously cycled the experimental knee between 40° and 110° of flexion once every 45 seconds, for 8 hours per day for 2 weeks, after which they were returned to ad libitum cage actvities for the remainder of the experiment. Eighteen animals from the two groups were killed at 6 weeks postoperatively, 18 more at 12 weeks, and the remaining 8 animals were killed at 26 weeks postoperatively.

The repaired site was assessed grossly to determine whether the defect was filled with firm, cohesive neocartilaginous tissue (Amiel et al. 1985). Samples with defects that fit this criteria were labelled as biologically acceptable and were then subjected to mechanical tests. Fresh rib perichondrium, as well as normal articular cartilage obtained from contralateral medial femoral condyles of 6-week rabbit knees, was also tested.

Mechanical tests. A dynamic shear-testing apparatus similar to Miles (1962) was used to measure the viscoelastic properties of the neocartilage. The apparatus consisted of two parallel plates between which the cartilage specimen was placed (Figure 2). One plate was attached to a piezoelectric driver (shear generator), which generated the

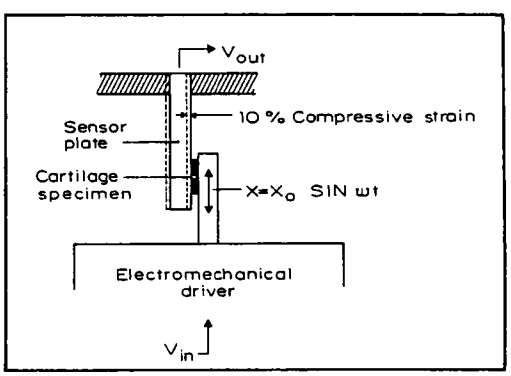

Figure 2. Diagram of the dynamic shear testing apparatus. A 10 per cent compressive strain was applied to hold the test specimen in place between the shear generator and the sensor plate. The sinusoidal motion of 100–600 Hz was applied to the specimen, and the resulting motion in the specimen was recorded by the ceramic sensor plate.

sinusoidal shearing motion. The other plate (sensor plate), which was made of polarized ceramic barium titanate, was fixed to the apparatus to detect the stress produced on the specimen. Due to the sensitivity of the measurements, it was necessary to eliminate vibration. This was accomplished by mounting the entire test apparatus on a 50-kg metal slab, which was in turn set on top of a vibration isolation table.

The system was calibrated by using a stainless steel ring with known dimensions. The ring was interposed between the shear generator and the sensor plate. Sinusoidal motions with a small amplitude ($\sim$0.05 μm) were applied to the ring, and responses over the frequency range of 25–800 Hz were obtained (Figure 3). The ratio of output voltage to input voltage was found to be linearly related to frequency over the range of 100–600 Hz. In this frequency range, a frequency-dependent constant was calculated by using the following equation (Miles et al. 1965):

$$K(\omega)=R(V_{in}(\omega)/V_{out}(\omega)) \quad (1)$$

where $V_{in}(\omega)$ is the input voltage to the shear generator, $V_{out}(\omega)$ is the corresponding output voltage recorded by the sensor plate, ω is the frequency, and R is the rigidity of the ring given by

$$R=4.48\ Ebt^3/d^3 \quad (2)$$

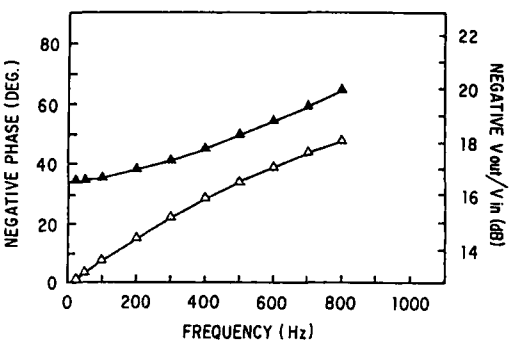

Figure 3. Calibration curves for the dynamic shear testing. Amplitude ▲ and phase delay △ are plotted as functions of frequency. In both cases, linear relation was observed in the frequency range between 100–600 Hz.

where E is the elastic modulus of the stainless steel, and d, b, and t are the diameter, width, and thickness of the ring, respectively. The calibration constant $K(\omega)$ is later used to compute the complex shear modulus of the test specimen.

Sample preparation and testing. Biologically acceptable neocartilaginous tissue was harvested from the rabbit femoral condyle. For the dynamic shear test, specimens of a uniform thickness 200–300 μm were obtained from the surface layer of the neocartilage and were cut into 2 mm × 3 mm cross sections. Precise dimensions of each specimen were measured by using a cathetometer and a thickness micrometer (Woo et al. 1976). The repeatability of these measurements was within ± 15 μm.

The test specimen was placed between the shear generator and the sensor plate. A compressive strain of 10 per cent was applied to hold the specimen in place (Figure 2). A small input sinusoidal wave with an amplitude of 0.05 μm, which corresponded to a shear strain of approximately 0.0002, was applied. The frequencies used ranged from 100 Hz to 600 Hz (at 100 Hz intervals). Both the input and output voltages were recorded using a gain-phase meter, which computed the amplitude ratio (V_{out}/V_{in}) and the phase lag between the two signals automatically. The magnitude of the complex shear modulus for the specimen $|G^*(\omega)|$ was then calculated by the equation (Miles et al. 1965):

$$|G^*(\omega)|=K(\omega)\ [(V_{out}(\omega)/V_{in}(\omega)](h/A) \quad (3)$$

where h is the distance between the shear generator and the sensor plate, and A is the cross-sectional area of the test specimen. The complex shear modulus is an important parameter describing the properties of a viscoelastic material, in this case, the solid matrix of the regenerated neocartilage. In the case of an isotropic elastic material, the shear modulus can be converted into the elastic modulus. To ensure measurement of the true tissue properties, the test was repeated three times for each specimen and the average results were taken. The variation of the measured data for each specimen was within 10 per cent. Care was taken so that the specimen was kept wet with normal saline at all times.

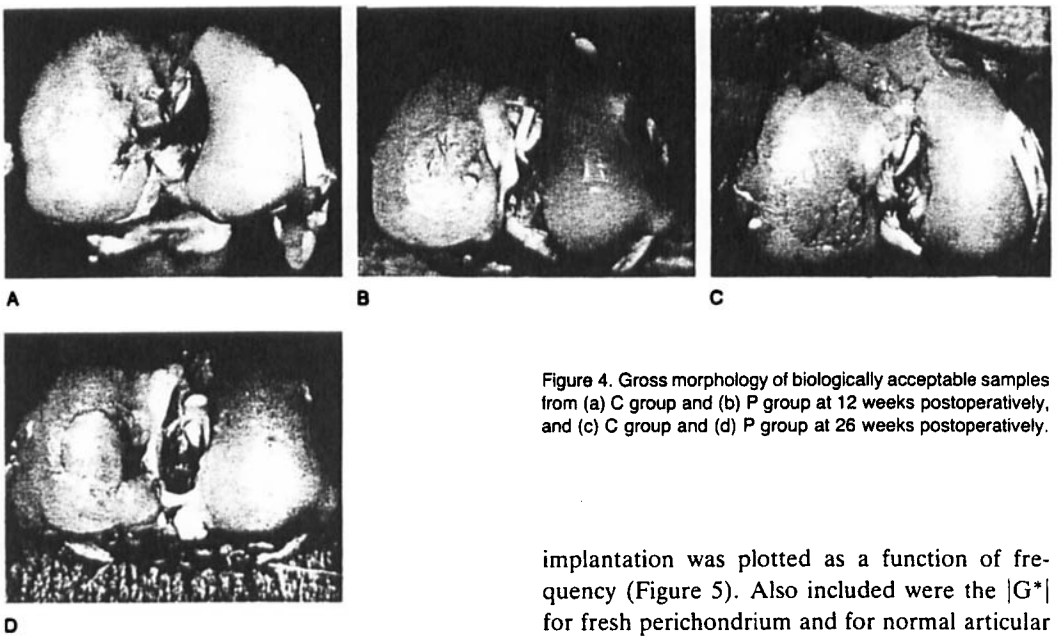

Figure 4. Gross morphology of biologically acceptable samples from (a) C group and (b) P group at 12 weeks postoperatively, and (c) C group and (d) P group at 26 weeks postoperatively.

Results

A total of 24 experimental samples met the criteria for biological acceptance. The ratio of biologically acceptable joint surfaces to total number of animals killed at 6, 12, and 26 weeks was 4/9, 5/9, and 3/4, respectively, and for the C group, and 4/8, 5/8, and 3/4, respectively, for the P group. A condylar fracture occurred once and there was one infection. Failure of the graft to generate sufficient cartilage to completely fill the defect was the most common reason for samples not being acceptable. In a few cases the autograft became loose, generating a polyp of cartilage. In both the C and the P groups, successful experimental joint surfaces were filled with cartilaginous tissue by 6 weeks. The samples from the P group appeared furrowed and less smooth than that from the C group, and their color was closer to that of the surrounding cartilage (Figure 4). In most cases the P samples also showed a greater degree of confluence of the neocartilage with the neighboring tissue than did the C samples despite their more ruffled appearance. However, osteophytes around the femoral condyles were also more common in neocartilage from the P group.

The magnitude of the complex shear modulus $|G^*|$ for neocartilage after 6, 12, and 26 weeks of implantation was plotted as a function of frequency (Figure 5). Also included were the $|G^*|$ for fresh perichondrium and for normal articular cartilage. It can be seen that the perichondrium had a much lower $|G^*|$ (with a very small SEM) than the normal articular cartilage, although the $|G^*|$ for both increased with increasing frequency. For the neocartilage from the experimental knee, temporal changes in $|G^*|$ were observed. At 6 weeks the $|G^*|$ for the C specimens was slightly higher than that for the perichondrium (Figure 5), while the $|G^*|$ for the P group was approximately three times higher than that for the C specimens. At 12 weeks, the $|G^*|$ for the C specimens approached that for the P specimens, as the changes in $|G^*|$ for the latter between 6 and 12 weeks were minimal. With time the viscoelastic properties of the neocartilage tissues in both treatment groups continued to improve, approaching that of normal articular cartilage.

While time had an effect on the $|G^*|$ of the neocartilage ($P < 0.05$), treatment modality did not ($P < 0.05$). Subsequent comparisons of the group means by using the Bonferroni test indicated that there was no difference between any of the groups.

Discussion

Comparison between viscoelastic behaviors of arthroplasty surface and normal articular cartilage was first studied by Coletti et al. (1972). Since then, only limited attempts have been made to evaluate the viscoelastic properties of repaired

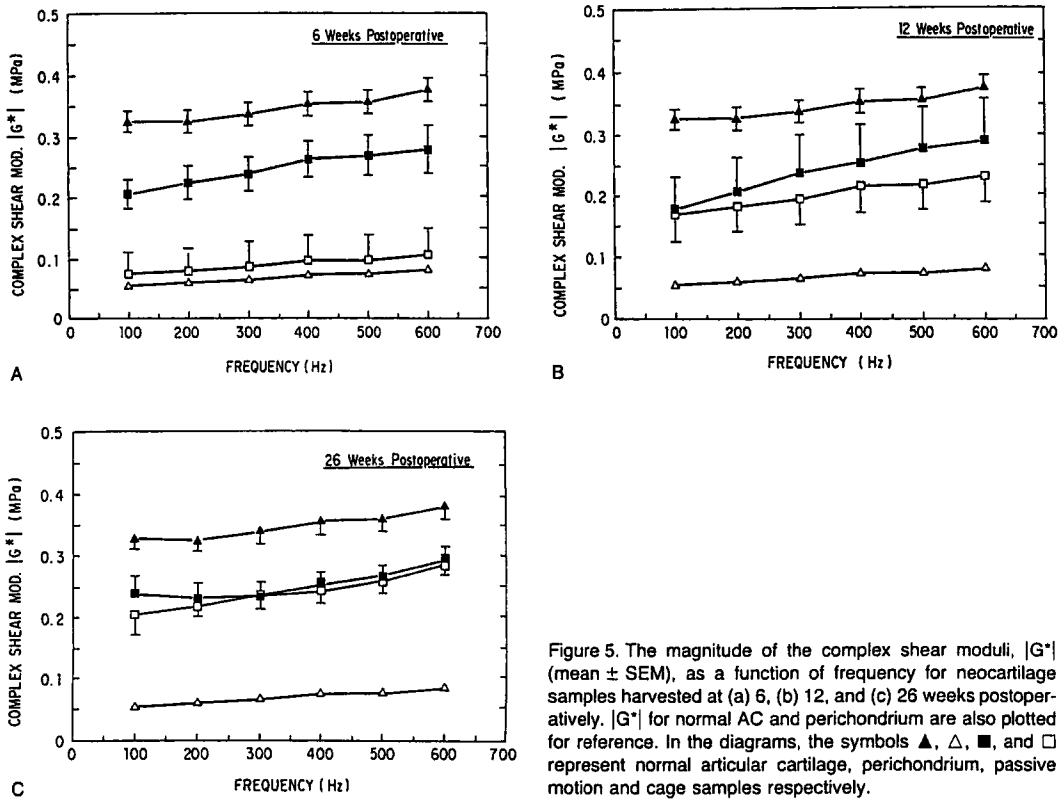

Figure 5. The magnitude of the complex shear moduli, |G*| (mean ± SEM), as a function of frequency for neocartilage samples harvested at (a) 6, (b) 12, and (c) 26 weeks postoperatively. |G*| for normal AC and perichondrium are also plotted for reference. In the diagrams, the symbols ▲, △, ■, and □ represent normal articular cartilage, perichondrium, passive motion and cage samples respectively.

articular cartilage. Whipple et al. (1985) studied repaired cartilage in a swine model. Their results, however, are limited to regenerated fibrocartilage. Other studies including a recent report by O'Driscoll et al. (1985) lack mechanical data for comparisons. It is believed that the present study is the first to measure the viscoelastic properties of hyaline-like cartilage regenerated at the articular surface.

The measured values for the complex shear modulus for both normal and regenerated cartilage were within the range of shear modul reported for normal rabbit articular cartilage (Parsons & Black 1977, Hoch et al. 1983), indicating that our results obtained in this study were reasonable. Using the dynamic shear testing apparatus, the properties of the neocartilage generated from the perichondrial autograft were found to resemble those for normal articular cartilage as postoperative time increased. These results correlated well with previous biochemical data from our laboratory for the early postoperative time period (Amiel et al. 1985). At 6 and 12 weeks postoperatively, the neocartilaginous tissues were found to contain approximately 55 and 65 per cent of type II collagen in both C and P groups, respectively, and the total collagen contents were 532 ± 38 and 566 ± 40 mg/g of dry tissue, respectively (Amiel et al. 1985). The increase in collagen content with healing time corroborated the increase in complex shear modulus. Comparatively, the increase in complex shear moduli for the neocartilage was more rapid than the increase in collagen content. This may have been related to microstructural changes occurring during maturation of the newly grown tissue matrix.

In the early stage, the gross and histologic data (Amiel et al. 1985) for the neocartilage suggested that passive motion treatment may not have had a beneficial effect in terms of the biological acceptability of the repair site. Yet, neocartilage from the acceptable samples in the passive motion group was shown to have mechanical properties closer to those of normal articular cartilage than did those from the cage group. The difference may be due to the fact that passive motion applied

postoperatively may injure the progenitor cells of the autografts, thus resulting in early failure and/or graft detachment, Yet, if the graft survives, circulation of the synovial fluid and small compressive force on the graft generated by passive joint motion may stimulate the growth of the neocartilage.

Our results suggest that the perichondrial autograft may be a source of material for replacement of large cartilage defects. The morphological, biomechanical and biochemical properties of the neocartilage from the autograft continue to improve with time. At 26 weeks, the viscoelastic properties of the neocartilage approach the properties of normal articular cartilage. Long-term survivability will, however, need to be evaluated.

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Acknowledgements

Support from NIH Grants AM28467 and AM07484 is gratefully acknowledged.