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ORIGINAL STUDY

Static indentation test for neocartilage surface hardness in repair of periosteal articular cartilage defects

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The objective of this study was to determine whether hardness of the superficial layer is a useful parameter to characterise cartilage produced by periosteal neochondrogenesis, using rabbit knee lesions as a model. A cartilage defect was created in the right hind knee of anesthetised young adult rabbits, and the defect was then covered with an autologous periosteal graft. At one and eight months post-surgery, rabbits were euthanised, and the articular cartilage lesion sites were evaluated for the histological parameters in a modified O'Driscoll scale, which is the current 'gold standard' for new cartilage properties. In addition, a static indentation test was performed, using a Shore-A sclerometer to measure surface hardness of the new cartilage. The hardness values had a statistically significant, positive correlation with the O'Driscoll parameters. This combination of a biomechanical measure and the O'Driscoll scale provided a more definitive indicator of graft quality. The results suggest that a hardness test with some type of sclerometer should be included in the functional characterisation of all engineered or grafted neocartilage.

Keywords : periosteum ; cartilage ; hardness ; neochondrogenesis ; periosteal arthroplasty.

INTRODUCTION

Hardness tests (indentation tests) have been used to study the mechanical properties of many biological tissues, especially normal (*11, 21, 23*) and damaged (*18*) articular cartilage *in situ*. Hardness measurements have shown that visibly normal-appearing areas of cartilage do not necessarily have normal mechanical properties. Some of the mechanical characteristics of neocartilage, such as tensile properties of the external surface, might be significantly inferior to those of normal articular cartilage.

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Table I. — O' Driscoll's neocartilage evaluation scale (modified by Chang 1999)

Particulars of study	Score
Filling of defect	
125% (Above articular surface)	1
100% (At articular surface)	0
75% (At osteochondral junction)	1
50% (At subchondral level)	2
25% (At bone)	3
0% (None)	4
Nature of predominant tissue	
Mature hyaline cartilage	0
Immature hyaline cartilage	1
Undifferentiated mesenchymal cells	2
Fibrous tissue	3
Reconstitution of osteochondral junction	
Yes	0
Almost	1
Not closed	2
Bonding to adjacent cartilage	
Bonded at both ends	0
Bonded at one end or partially at both	1
Not bonded	2
Matrix staining	
Normal	0
Reduced staining	1
Significantly reduced	2
Faint staining	3
No stain	4
Cells	
Normal	0
Diffuse Hypercellularity	1
Cloning	2
Hypocellularity	3
Structural integrity	
Intact	0
Partially disrupted	1
Completely disrupted	2
Biomechanical control (hardness test)	
Normal Hardness (>90)	0
Abnormal (70-90)	1
Resilient (< 70)	2

However, to our knowledge, hardness tests have not been applied to the cartilage-like tissue produced by periosteal neochondrogenesis (referred to as "repair tissue").

Periosteal transplantation has become a well-established method for replacing, or partially regenerating articular cartilage (*8*). Some, but not all, characteristics of the repair tissue produced by periosteal neochondrogenesis are similar, and sometimes identical to those of normal hyaline cartilage (*5, 7-10, 12, 20, 24, 28*). To date, most of the analyses of neocartilage have been observational, at the macroscopic, histological, and ultrastructural levels. The most widely used scale defining quality of the new tissue is the one developed by O' Driscoll (26), and later modified by others, including Chang *et al* (*6*), and Carranza-Bencano *et al* (*5*) (table I). However, a reliable, objective, quantitative method is needed to assess the mechanical properties of the newly formed chondrocyte-matrix structure, including surface hardness, tensile strength, and the ability to bear the high compressive loads generated in synovial joints. Toward that end, in this paper we describe a simple, reproducible indentation test that provides a quantitative surface hardness measurement on cartilage. This parameter was shown to have a statistically significant, positive correlation with the established histological quality indicators. The test may be used with cartilage produced by tissue engineering, as well as by periosteal transplantation, and it allows rapid comparison of the mechanical properties of these repair tissues and normal hyaline cartilage.

MATERIALS AND METHODS

All experiments took place in the National Technical University in Athens. Protocols for the surgical procedures, and pre- and post-operative animal care were reviewed and approved by our Institutional Animal Care and Use Committee (license no. 1943/9/9/2001), and were documented in compliance with the guidelines of the Greek National Institute of Health, and with the regulations on the care and use of laboratory animals.

Sixty 12-week old New Zealand rabbits (weight $\geq$ 2.5 kg) were used. General anaesthesia was induced by intraperitoneal injection of a mixture of ketamine (40 mg/kg) and xylazine (6 mg/kg). A cartilage defect $0.5 \times 0.5 \times 0.2$ cm deep was created in the medial femoral condyle of the right hind knee of each rabbit. Each defect was then covered with a periosteal graft harvested from the upper third of the animal's tibia through the same incision. The graft, with its cambium layer facing the joint, was sutured at the lesion site with 6.0 PDS sutures and fibrin glue (a 1:1 mixture of rabbit fibrinogen and bovine thrombin). The glue was used to fix the

Table II. — Scoring of the specimens according to the modified O' Driscoll scale

A : Assessment after 1 month

Rabbit	1	5	10	11	12	15	18	21	22	23	24	25	26	32	33	34	35	39	40	41	42	43	44	45	46	47	48	49	50	56
Filling of defect	3	4	1	1	2	1	1	1	4	1	4	4	3	1	4	1	2	4	2	2	1	1	2	1	2	1	1	1	1	4
Nature of predominant tissue	2	2	3	2	2	3	2	1	3	1	3	3	3	3	2	3	3	2	2	3	3	2	2	1	2	1	2	2	2	2
Reconstitution of osteochondral junction	2	2	2	2	1	3	3	1	2	1	2	2	2	2	2	2	2	2	1	2	3	2	2	1	2	1	3	2	2	2
Bonding to adjacent cartilage	1	1	1	1	1	2	2	1	1	1	2	2	2	2	1	1	1	2	1	1	2	1	1	1	2	1	2	2	2	1
Matrix staining	3	3	3	1	1	2	2	1	2	2	2	2	2	3	3	3	2	2	2	1	2	2	1	1	2	1	2	1	2	2
Cells	3	3	3	2	1	1	1	1	1	1	1	1	2	3	3	3	3	2	1	3	1	2	3	0	2	1	1	2	2	1
Structural integrity	1	1	2	2	1	1	1	1	2	1	2	2	2	2	1	2	2	2	1	2	1	2	2	1	2	1	1	1	1	2
Biomechanical control hardness test	1	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	1	2	1	2	2	2	2
Total	16	19	17	8	13	15	14	9	17	10	18	18	18	18	19	17	17	18	16	11	16	14	15	7	16	8	14	13	14	16

B : Assessment after 8 months

Rabbit	3	4	6	7	8	9	13	14	16	17	19	20	27	28	29	30	31	36	37	38	51	52	53	54	55	57	58	59	60	2
Filling of defect	1	1	1	0	1	1	0	1	2	1	1	1	2	3	3	2	4	4	3	3	1	2	4	1	1	1	1	0	1	1
Nature of predominant tissue	1	2	2	1	1	1	1	3	2	1	0	2	2	1	2	2	2	3	3	2	1	2	3	2	3	3	2	2	3	2
Reconstitution of osteochondral junction	2	2	1	0	0	2	1	2	2	0	2	2	2	1	2	2	2	3	3	2	2	1	2	1	2	1	1	1	2	1
Bonding to adjacent cartilage	1	1	2	1	0	2	0	2	0	1	2	1	1	2	1	1	2	2	2	1	2	2	2	1	2	1	1	1	2	1
Matrix staining	1	1	2	1	1	1	1	2	1	1	3	2	2	2	2	2	3	2	2	1	1	2	3	2	1	1	3	2	1	1
Cells	1	2	2	1	1	2	0	1	0	0	1	2	1	2	1	2	3	2	2	1	2	2	2	2	1	2	2	2	1	1
Structural integrity	1	1	1	1	1	1	0	2	1	1	1	1	2	2	2	2	2	2	2	1	2	2	2	1	2	2	1	1	1	1
Biomechanical control Hardness test	2	2	2	1	0	2	0	2	2	1	1	2	2	2	2	2	2	2	1	2	2	2	2	2	2	1	1	2	2	2
Total	10	12	13	6	5	12	10	15	10	6	11	13	14	16	15	15	20	20	18	13	13	16	20	12	14	12	12	11	11	10

periosteum to the adjacent cartilage, and not to the bony bottom of the defect. The incision was then closed with Monocryl 4.0 (subcuticular). All animals were allowed normal mobility immediately after they recovered from anesthesia. No post-operative complications occurred.

Thirty knees with neocartilage, and 30 control knees from the same animals were assessed at one month post-surgery, and an identical group was examined at eight months following surgery (table II A, B).

At one and eight months after the surgery, the rabbits were euthanised by intravenous administration of an overdose of thiopental. The operated right hind knee joint was then excised and carefully cleaned of all neighbouring tissues. The left hind knee joint was also removed and cleaned, to serve as a control. The site of the articular cartilage lesion was examined macroscopically, microscopically, and histologically for indicators in the O'Driscoll scale modified by Chang *et al* (6) : percentage of hyaline articular cartilage (nature of predominant tissue), structural properties (including surface regularity, structural integrity and bonding of graft to adjacent cartilage), occurrence of degenerative changes in cells or adjacent cartilage, bonding of repair cartilage to *de novo* subchondral bone, and extent of staining with Safranin O (fig 1 a-e), (table II).

To augment the histological examination, the specimens were tested biomechanically. An indentation test was performed using a Shore-A sclerometer (fig 2 a, b). This instrument (also called a durometer) consists of a small hand-held gauge with an outer cylinder attached to a disc called a presser foot. Inside the cylinder is an indentation tip, with a flat end of approximately 15 µm diameter, which contacts the surface to be tested. The other end of the indentation tip is linked to the gauge by a high-precision calibrated spring. The device is placed on the material to be tested. Gentle hand pressure drives the indentation tip in the vertical direction. The tip pushes on the sample surface, while a counterforce is exerted by the presser foot (fig 2 b, fig 3). The gauge shows the force needed to indent the sample surface. Small blocks of samples with known hardness are used to calibrate the device. Shore hardness is a measure of the resistance of material to indentation. The higher the number, the greater the resistance. If the indenter comes in contact with a very soft sample a reading of 0 is obtained ; if it comes into contact with a very hard sample, a reading of 100 results. The reading is dimensionless.

The indentation measurements were carried out at room temperature (20°C). Each specimen was anchored in a clamp, and the indenter and the presser foot were brought into contact with the surface of the cartilage (fig 3). A load was applied, held for 10 seconds, and then removed. The sclerometer was first calibrated on normal articular cartilage by taking three measurements at exactly the same site in the left knee as in the operated knee. The mean value was then accepted as the hardness of normal cartilage. Hardness values of all normal cartilage specimens ranged between 90 and 100 (table III a, b). Similarly, three measurements were made at the same location on each cartilage transplant site in the test knees at 10 minute intervals, and the mean value was calculated (table III c). In order to match the diagnostic scores in the Driscoll/Chang evaluation scale in table I, we added the Shore surface hardness score with less hardness represented by higher point values. We arbitrarily assigned a score of 0-2 as follows : normal cartilage hardness (hardness values of 90-100) was scored as zero (0), hardness measuring 70-90 was scored as one (1), and hardness measuring < 70 was scored as two (2).

Statistical analyses

Analyses were performed (a) to determine the variation in the O'Driscoll histological grading scale, which is the benchmark for this study, (b) to assess the accuracy and reproducibility of the hardness value, which was the new variable, (c) to test the correlation between the hardness value and the O'Driscoll scale, and (d) to establish whether the O'Driscoll scale alone, or combined with the hardness value, is more consistent with the variance in the experimental results.

To determine the variation in the modified O'Driscoll histological grading scale, the knee joints with the transplants were evaluated independently by two experienced orthopaedic surgeons and a pathologist. The outcomes were analysed using a Wilcoxon rank sum test. Accuracy and reproducibility of the hardness values and O'Driscoll scale values were determined at one month and at eight months post-operatively, by pairwise *t*-test. Pearson correlation coefficients for the hardness value *versus* the O'Driscoll scale were calculated for the results obtained with the specimens one month and eight months post-operatively, to establish whether the new variable positively correlated with the O'Driscoll scale.

Two statistical methods were used to establish whether the O'Driscoll scale alone, or combined with the hardness value, was more consistent with the variance in the experimental results. The first, Cronbach's alpha, is an index of reliability (4) associated with the variation accounted for by the true score of the "underlying construct". In this case, the construct was the new

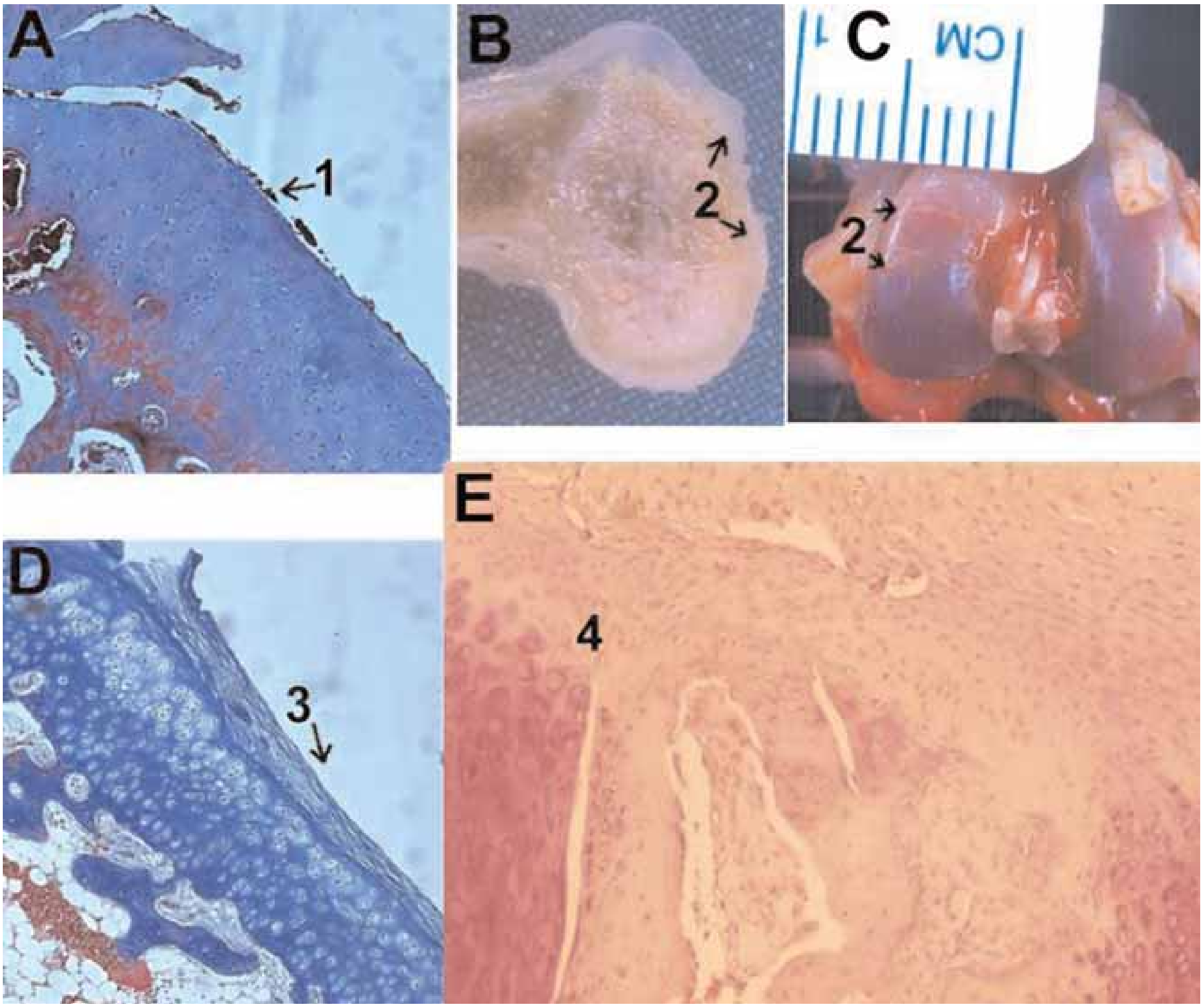

Fig. 1. — **a.** Repair tissue produced by periosteal neochondrogenesis. (Number 1 indicates the Indian ink grains used to spot the area of the repair tissue.) (Masson-Trichrome × 40) ; **b.** Macroscopic sagittal section of a knee with partial regeneration of the lesion site 8 months following periosteal transplantation. (Number 2 shows the lesion site) ; **c.** Macroscopic result at 8 months post-op, where the lesion is fully covered with the repair tissue ; **d.** Histological result of a knee, 8 months post-op with a satisfactory outcome.(Number 3 indicates the parallel fibers of connective tissue on the surface of the repair tissue which is the periosteum) (Masson-Trichrome × 60) ; **e.** Histological result of a knee, 1 month post-op. (Number 4 shows the section of the normal cartilage and the area between the normal cartilage and the repair tissue) (H&E × 60).

variable, hardness. The second method, factor analysis, was used to determine how much of the total variance in the experimental data could be accounted for by hardness, or any of the other variables that comprise the O'Driscoll scale.

All statistical analyses were done with the SPSS 12.0 software package. Statistical significance was defined as $p \geq 0.05$ (*32*).

RESULTS

In the knees tested, the grafted tissue was found to be mechanically inferior to the normal cartilage in the control knees : hardness 50-70, relative to 90-100 for the controls (table III). The Shore sclerometer was easy to use, and the results were

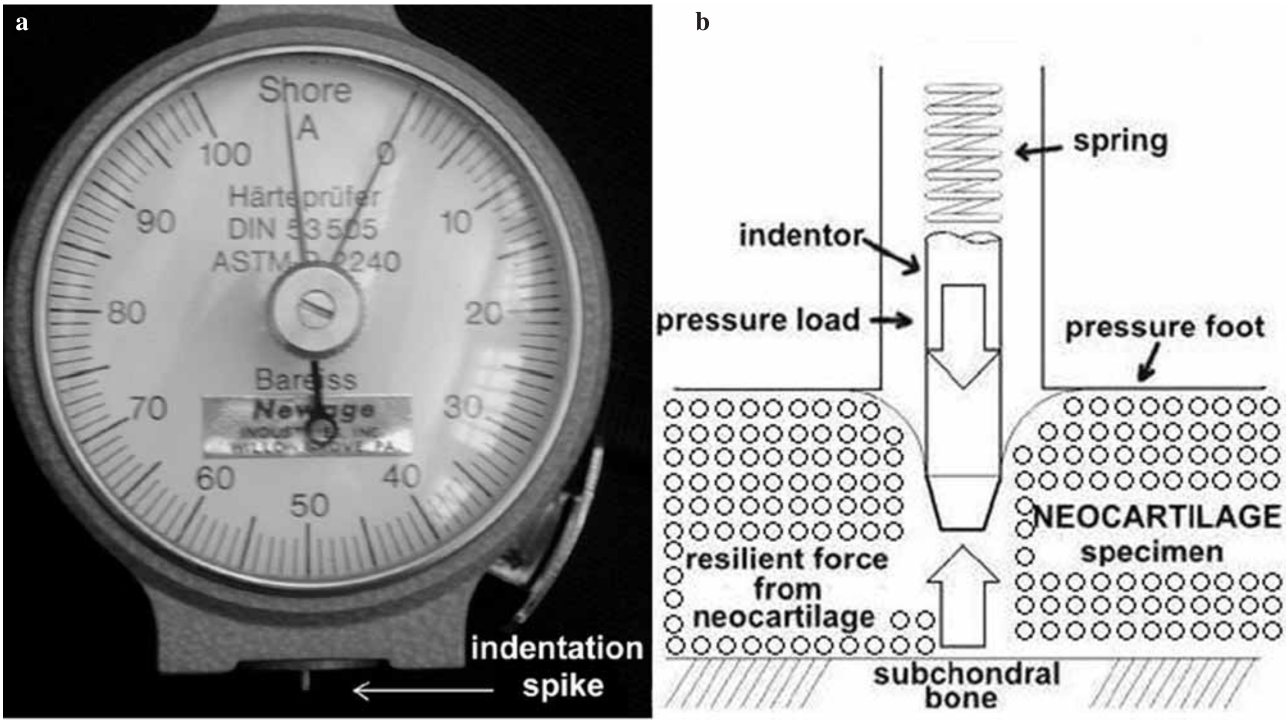

Fig. 2. — **a.** The "Shore A" sclerometer used in our study ; **b.** Representation of an indenter with the pressure foot applied to a thin layer of neocartilage. **Mechanism of the hardness measurement** : **T**he indenter gives a distortion onto the surface of the sample with the presser foot, and pressure produced by the spring load. The "hardness" means the depressed amount of the indenter at the time when the resilient force becomes equal to the pressure load. Thus, the value will consequently reflect the "physical amount" with no unit.

very reproducible. However, two issues had to be addressed before it could be used reliably. These were the geometry of the indentation tip, and determination whether penetration had occurred. Several analyses of indentation methods have been published (*11, 16, 17, 30*), but none examined the hardness of the outer layer of neocartilage that develops in a graft. Our tip, as described in Materials and Methods, was selected for its ability to indent the cartilage by 0.3 mm, without puncturing the surface.

If the indenter tip punctures the surface of the cartilage, the indentation force measurement is invalid. Therefore, we conducted a pilot study with 12 specimens, in which a small amount of Indian ink was applied after the indentation. If the cartilage surface was penetrated, the India ink would infiltrate the tissue. This could be observed easily under a dissecting microscope. Using this technique, we found that our indenter did not penetrate any of the specimens.

The histological results showed that the repaired tissue consisted of hyaline-like cartilage in most of the areas examined (Fig 1a-c, table II). At the end of one month after surgery, the O'Driscoll scale parameters for the grafted cartilage that most resembled normal cartilage (table I) were the type of predominant tissue (p = 0.019), number of cells (chondrocytes) in matrix (p = 0.014), matrix staining (p = 0.030) and the total score (p = 0.011). In the eighth month post-surgery test group, additional properties of the grafted cartilage correlated more closely with those of normal cartilage. These were : reconstruction of the osteochondral junction (p = 0.007), bonding of neocartilage to adjacent cartilage (p = 0.017), predominant tissue type (p = 0.006), and the total score (p = 0.029). All the above mentioned parameters were evaluated with the Mann Whitney U-test.

Cronbach's Alpha statistic can be used to estimate the reliability of data when there are two or more comparable scores per subject. Scales such as

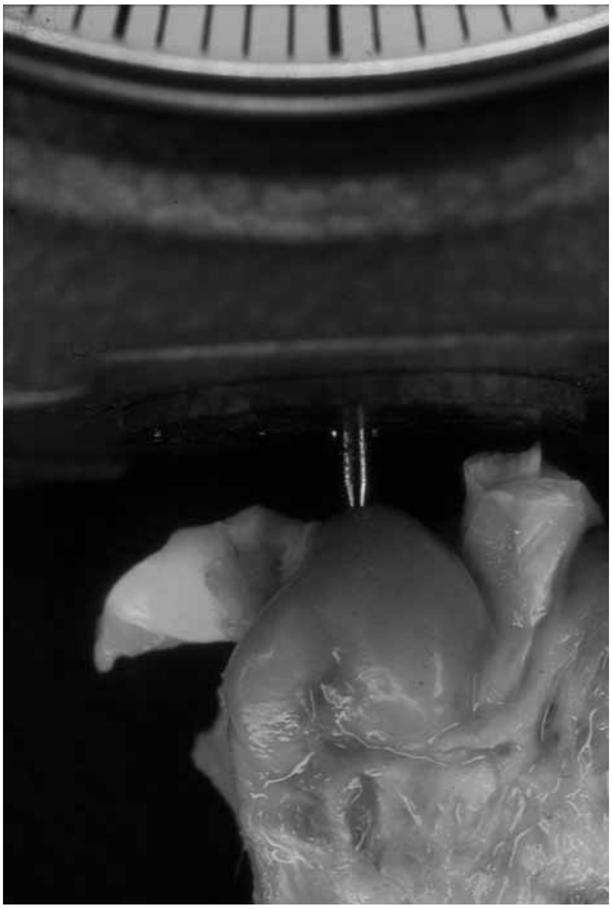

Fig. 3. — Indentation test of the repair tissue (neocartilage) following autologous periosteal transplantation in the rabbit femoral condyle, where the indenter comes in contact with the examination site.

the O'Driscoll are composed of criteria that all measure independent characteristics of one thing - in this case, normal cartilage. This implies that all of the criteria should be correlated with one another. In statistical terms, the variance of the sum of a set of independent variables is the sum of their individual variances. If the variables are positively correlated, the variance of the sum will increase (*4*). When all criteria in the scale correlate perfectly, Cronbach's alpha = 1.0. When there is no correlation among the variables, alpha = 0. Thus, this statistic is a measure of the internal consistency of the scale.

The Shore hardness values had good Pearson correlation coefficients with all of the parameters in the O'Driscoll scale, except "reconstruction of the osteochondral junction" and correlation with the latter improved with the experimental values obtained at eight months post-surgery (table IV). When the hardness values were combined with the O'Driscoll scale, the Cronbach's alpha increased from 0.7532 to 0.8016 for the one month post-surgery data, and alpha increased from 0.828 to 0.852 with inclusion of the data from eight months post-surgery (table V). Thus, inclusion of the Shore hardness increased the internal consistency and reliability of the overall graft evaluation system. Factor analysis of the data from one month post-surgery, showed that the total variance explained by the O'Driscoll scale was 78.5% without the hardness parameter, and 83.2% after the hardness was included. With the data from eight months post-surgery, the variance explained by the O'Driscoll scale remained about the same : 70.5% and 68.1% with, and without the hardness parameter, respectively.

DISCUSSION

Periosteal transplantation to repair cartilage defects was first described by Rubak *et al* (*28, 29*) and subsequently studied by many others (*2, 5, 8, 14, 15, 25-29, 33, 35*) at the experimental level, and as in clinical practice (*1, 13, 19, 20, 23, 24*). Autologous periosteal graft transplantation has shown promising results in treating defects of articular cartilage. Tissue engineering technology is also advancing rapidly as a source of new cartilage for grafting. With both techniques it is now possible to produce new cartilage that is histologically almost indistinguishable from normal hyaline-like cartilage.

All specimens were evaluated with the standard O'Driscoll scale (*26*) as modified by Chang *et al* (*6*) (table I), because it is simple, reproducible, and can be applied easily by scientists and clinicians in different specialties. The O'Driscoll scale and its more recent modifications have been excellent objective tools for histological comparison. However, mechanical properties of the newly formed tissue must also be characterised and meet

Table III. — Hardness test [grafted periosteum VS control (normal knees)]

A : Assessment after 1 month

Rabbit	1	5	10	11	12	15	18	21	22	23	24	25	26	32	33	34	35	39	40	41	42	43	44	45	46	47	48	49	50	56
Test knees (periosteal graft)	76	62	54	65	78	66	65	68	54	62	60	55	58	54	60	54	57	59	77	53	62	57	84	84	65	76	67	60	57	58
Control knees (normal cartilage)	98	96	96	98	92	95	94	99	93	95	97	96	92	94	92	96	98	95	97	94	95	92	95	98	93	93	98	96	92	94

B : Assessment after 8 months

Rabbit	2	57	3	4	6	7	8	9	13	14	16	17	19	20	27	28	29	30	31	36	37	38	51	52	53	54	55	58	59	60
Test knees (periosteal graft)	64	75	57	58	61	80	92	57	90	68	70	82	78	66	53	56	55	52	57	53	75	65	63	56	52	63	60	74	58	63
Control knees (normal cartilage)	96	96	94	97	97	98	94	94	94	96	96	98	96	95	95	93	93	99	95	94	96	94	95	98	94	97	97	95	98	97

C : Hardness averages of the two groups

	Mean	**Std. Deviation**	**Std. Error Mean**
Grafted knees after 1 mth	63.57	9.069	1.656
Control knees (normal cartilage)	95.10	2.155	.393
Grafted knees after 8 mths	65.10	11.118	2.030
Control knees (normal cartilage)	95.70	1.643	.300

Controls in both groups (1m, 8m) : $p < 0.0001$.

Table IV. — Assessment of internal consistency with Cronbach alpha 1 and 8 months post-operatively
(Number of items 8, number of Cases = 30.0)
Item-total Statistics

items	1 month overall Cronbach alpha	Alpha if Item Deleted (1 month)	8 months overall Cronbach alpha	Alpha if Item Deleted (8 months)
Filling of defect	0.8016	.7953	0.8521	.8233
Nature of predominant tissue		.7289		.8416
Reconstitution of osteochondral junction		.8164		.8408
Bonding to adjacent cartilage		.7943		.8191
Matrix staining		.7501		.8546
Cells		.8086		.8361
Sructural integrity		.7742		.8281
Hardness		.7532		.8280

Cronbach Alpha = Reliability Coeficient.

Table V. — Correlations between the modified O'Driscoll's scale and the hardness value in the grafted knees (N = 30)

Hardness after grafting		Filling of defect	Nature of predominant tissue	Reconstitution of osteochondral junction	Bonding to adjacent cartilage	Matrix staining	Cells	Structural integrity
at 1 month	r p value	0.603* < 0.001	0.644* < 0.001	0.084 0.659	0.363* 0.049	0.658* < 0.001	0.425* 0.019	0.420* 0.021
At 8 months	r p value	0.678* < 0.001	0.343 0.064	0.427* 0.019	0.548* 0.002	0.239 0.203	0.427* 0.018	0.503* 0.005

r = Pearson's coefficient
*statistically significant correlation.

standards. Walker (*34*) cautioned that macroscopic or microscopic analysis should always be accompanied by biomechanical analysis, as the ability of the tissue to withstand loading (especially in the knee) may be more critical than its morphological appearance. Rather than create a totally new mechanical evaluating system that may not integrate well with the histological criteria, we attempted to add a biomechanical parameter, the surface hardness, to the existing modified O'Driscoll scale.

In this study, our results showed that surface hardness of the repair tissue was significantly less than that of normal hyaline articular cartilage. This raises the questions of why repair tissue has less surface hardness, and how this limitation may be overcome.

The general answer to these questions is that there are physical, chemical and mechanical interactions between collagen and proteoglycans which are responsible for the mechanical properties of articular cartilage, and which are not yet fully elucidated. Cartilage has been characterised as a nonlinearly permeable, viscoelastic material, consisting of two principal phases : a solid organic matrix which is composed predominately of collagen fibrils and proteoglycan macromolecules, and a movable interstitial fluid phase, which is mainly water (*3, 22*). Water is mostly concentrated at the surface layers of the articular cartilage. When the cartilage is loaded by a compressive force, approximately 70% of the water is moved through the matrix, and this interstitial fluid movement is important in controlling the cartilage's mechanical behaviour. Proteoglycans also resist compression, both by Donnan osmotic swelling pressure mechanisms (pressures from 0.05-0.35 Mpa), and by adding to the bulk compressive stiffness of the collagen-proteoglycan solid matrix. Another possible explanation for the lower hardness of the neocartilage is that although in some areas there is hyaline-like cartilaginous structure, in other areas there are fibrous elements, and the repair tissue at those sites is mostly fibrocartilage. Unavoidably, the spots of soft fibrous tissue directly affect the hardness of the total cartilage surface.

This surface hardness difference between the normal and the neocartilage is a distinct limitation, because (as previously stated) the major functional role of articular cartilage is to bear weight (*10, 30, 34*). During joint articulation, forces at the joint surface may vary from almost zero to more than 10 times body weight (*22*). In addition, loading is more important than motion in maintaining cartilage properties (*34*). The hardness of the external surface is directly related to the ability to support a load. Loss of the surface layer hardness of the articular cartilage and the reduced ability to bear weight hinder the cartilage's normal lubricating and nourishment mechanisms, and trigger the degeneration process (*22, 34*). The less hard surface of the neocartilage indicates that we still lack some knowledge essential for producing repair tissue that is mechanically identical to natural cartilage.

Although many researchers and clinicians refer to the new tissue as "hyaline cartilage" (*5, 7-10, 12, 20, 24, 28*) we prefer the term "repair tissue". O'Driscoll, who has done extensive work in this area, coined the term "periosteal arthroplasty" (*24*), for what he foresaw as much wider use of autologous periosteal transplantation to treat large cartilage defects in the future. Chang *et al* (*6*) used pigs as a model system for periosteal cartilage transplantation, and modified the O'Driscoll histological grading scale to make a more versatile and easily reproduced evaluation protocol (*6*). Carranza-Bencano *et al* (*5*) recently demonstrated that it is possible to repair major full-thickness chondral defects in load-bearing areas of the rabbit leg, using periosteal grafts. Unfortunately, none of these studies included hardness of the surface layer, or any other mechanical property of cartilage. Our study was undertaken to develop a simple way to determine whether the repair tissue has weight-bearing capacity (is hard enough). In addition, we incorporated that measure in the standard evaluation scale for neocartilage, and validated the combined scale.

In statistical terms, scales such as O'Driscoll's are known as summated. Summated rating scales are composed of interrelated but independent parameters that measure underlying properties. As noted above, the scale is considered to be reliable only if it provides the same outcome over repeated, independent uses. The Pearson correlation coefficients, the Cronbach alpha statistic, and the separate factor analysis all support the relevance, internal consistency, and reliability of the scale.

The selection of an appropriate indenter probe tip geometry was essential for valid hardness tests. Many factors, including thickness of the sample, influence the measurement. A method similar to ours was published for measuring the thickness of bovine and canine cartilage specimens using a needle probe with reliable results (*17*). Hale *et al* (*11*) used an indenter with a spherical tip 1.0 mm in diameter to evaluate cartilage specimens. The force was measured when the indenter reached a depth of 0.3 mm. This corresponded to approximately 25% compression of the cartilage, which was typically 1.2 mm thick at the site. Unfortunately, these researchers did not develop a validated protocol to characterise the cartilage specimens as a whole. An indentation device with a conical tip for examination of cartilage fracture toughness was recently described by Simha *et al* (*31*). Their results also showed that indenters with small tip radii can be used on cartilage of minimal thickness (500 μm-1 mm). However, they did not incorporate their results into a validated evaluation scale.

In this study a specific device and protocol were developed for simple, reproducible, and reliable evaluation of the hardness of neocartilage. A more important aspect was the introduction of a new parameter into a standard evaluation scale, which previously had no criteria for mechanical strength. The mechanical properties are of primary importance to the clinician who hopes to treat patients using periosteal neochondrogenesis. It is also likely that our approach will be applicable for incorporating other biomechanical measures into the modified O'Driscoll, or similar summated rating scales.

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