Measurement of Articular Cartilage Surface Irregularity in Rat Knee Contracture

GUY TRUDEL, KO HIMORI, LOUIS GOUDREAU, and HANS K. UHTHOFF

ABSTRACT. ***Objective.*** To design novel quantitative methods to evaluate the irregularity of articular cartilage surface; and to apply these methods for assessment of surface irregularity in a rat knee contracture model.

Methods. A total of 117 rat knees were either immobilized or sham-operated and harvested after 2, 4, 8, 16, or 32 weeks, and 11 knees were not operated. Standardized histologic sections were digitized and the contours of femoral and tibial cartilage surfaces were delineated. The rates of change in cartilage contour were calculated. Rate of change above a defined threshold constituted surface irregularity.

Results. In non-operated knees, cartilage surface irregularity in femur and tibia amounted to 3.1 ± 0.5%. Immobilized knees showed significantly more irregularities than the sham-operated knees at all time points (2 weeks: 5.3 ± 0.6% vs 3.1 ± 0.4%; 4 weeks: 10.5 ± 0.9% vs 4.4 ± 0.9%; 8 weeks: 12.0 ± 1.8% vs 4.9 ± 0.2%; 16 weeks: 13.7 ± 2.0% vs 4.9 ± 0.4%; and 32 weeks: 13.8 ± 1.4% vs 3.4 ± 0.6%; all $p < 0.05$). No difference was observed between sham-operated and non-operated knees. Increasing duration of immobilization in weeks (t) significantly correlated with more surface irregularity, described by the logarithmic formula: % irregularity = 6.6 + 2.1 ln (t), ($F = 59.3$, $p < 0.001$). This formula showed that irregularity progressed rapidly after immobilization and plateaued after 8 weeks.

Conclusion. We designed methods to quantify cartilage surface irregularity and applied them to a contracture model. Cartilage surface irregularities appeared after 2 weeks of immobilization and progressed rapidly to plateau after 8 weeks. Combined with microscopic magnetic resonance imaging, this measurement of cartilage surface irregularity may constitute a sensitive tool to detect cartilage degeneration clinically. (J Rheumatol 2003;30:2218–25)

Key Indexing Terms:
CARTILAGE SURFACE — CONTRACTURE — DEGENERATION
IMMOBILIZATION — HISTOMORPHOMETRY

Immobility has been reported to cause multiple alterations to articular cartilage including increased hydration[1-4], increased and decreased proteoglycan (PG) synthesis[4-8], reduced concentrations of PG[1-3,6,8-12], reduced PG staining intensity[10,12], altered PG aggregate structure[1,3,8,11,13], increased collagen synthesis[14,15], maintained or elevated collagen content[3,11,14,15], decreased or unchanged thickness of cartilage[1,16,17], and increased or decreased number of chondrocytes[12,18-20].

From the Bone and Joint Research Laboratory, Department of Medicine; Division of Physical Medicine and Rehabilitation; Division of Orthopaedic Surgery, University of Ottawa; and the Rehabilitation Engineering Department, The Rehabilitation Centre, Ottawa, Ontario, Canada.

Supported in part by the Physicians of Ontario through the PSI Foundation and by the Canadian Institutes of Health Research.

G. Trudel, MD, MSc, Bone and Joint Research Laboratory and Division of Physical Medicine and Rehabilitation; K. Himori, MD, Bone and Joint Research Laboratory; L. Goudreau, Rehabilitation Engineering Department, The Rehabilitation Centre; H.K. Uhthoff, MD, Bone and Joint Research Laboratory and Division of Orthopaedic Surgery.

Address reprint requests to Dr. G. Trudel Department of Medicine, University of Ottawa, 505 Smyth Road, Ottawa, Ontario K1H 8M2, Canada.

Submitted August 28, 2002; revision accepted February 28, 2003.

In addition to these alterations, surface irregularity has consistently been observed in different experimental models and may constitute a good marker of cartilage degeneration[12,18,21-27]. Because of the importance of measuring cartilage degeneration not only after immobilization but also in osteoarthritis (OA) and rheumatoid arthritis (RA), attempts have been made to assess surface irregularity. In the past, qualitative histologic details of surface irregularity were reported with light microscopy[28], macroscopic and microscopic examination of India ink preparation[29], and polarizing microscopy and scanning and transmission electron microscopy[30-32]. More recently, Candolin, *et al*[22] studied surface irregularity of rabbit knee cartilage with scanning electron microscopy after 1 to 8 weeks of immobilization and assessed it semiquantitatively with a 5-grade scale based on appearance. Helminen, *et al*[23,24] and Jurvelin, *et al*[25] applied a similar categorical scale and reported cartilage surface irregularity in rabbit knees immobilized for 8 weeks. Unfortunately, these tools lack the discriminative power and quantitative properties that would enable the use of surface irregularity as an outcome measure of cartilage degeneration.

Technologies other than histology have been applied to the study of cartilage. Ultrasound could detect fibrillations in OA femoral head[33]. Unfortunately, in numerous models, including ours, immobilization does not lead to large fibrillatory changes. Laser based confocal and atomic force microscopy can capture cartilage roughness, but are restricted to *in vitro* samples and are not widely available[34,35]. Methods using mechanical or laser stylus instruments derive information on surface roughness in materials sciences[36,37]. However, there remains a need to design and apply precise and reliable methods to quantify cartilage surface irregularity *in vitro*. These methods could then be transposed to clinical application.

The purposes of this study were (1) to design novel quantitative methods to evaluate the irregularity of articular cartilage surface; and (2) to apply these methods to the assessment of surface irregularity in a rat knee contracture model. We hypothesized that immobility of a joint leads to articular cartilage surface irregularity that progresses over time.

MATERIALS AND METHODS

The Institutional Ethics Committee approved the protocol for these experiments. One hundred twenty-eight (128) adult male Sprague-Dawley rats were used. Seventy rats underwent unilateral extraarticular knee joint immobilization in flexion according to a described method[38]. Briefly, internal fixation was performed surgically with a plate and 2 screws (one inserted in the proximal femur and the other in the distal tibia) to avoid violation of any knee joint structures. Surgery was performed under halothane anesthesia and involved minimal dissection. For pre- and postoperative pain, buprenorphine 0.05 mg/kg was administered q 12 h for 48 h. The animals were allowed unlimited activity and free access to water and food. Forty-seven rats underwent sham surgery similar to the experimental group except that no internal fixation was left in place. In addition, 11 knees from non-operated rats were studied.

Tissue preparation and staining. The animals were sacrificed at one of 5 time points: 2, 4, 8, 16, or 32 weeks after surgical intervention. The knee joints and surrounding soft tissues were harvested, fixed in Bouin's solution or 10% formalin for 24 or 18 h, respectively, decalcified in 10% EDTA in Tris buffer 0.1 M (pH 7.2 adjusted with Tris base) at 4°C for 60 days, and embedded in low melting point paraffin (Labware, St. Louis, MO, USA). Seven micrometer serial sections obtained in the sagittal plane going through the medial mid-condyle level were used for this study (Figure 1). One slide from each specimen was selected to measure cartilage surface irregularity. All measurements were performed by one observer (KH) blinded to the group (immobilized, sham-operated, or non-operated) and the time after intervention (2, 4, 8, 16, or 32 weeks) of each slide. The code was broken only at the end of data harvesting.

Measurement of cartilage surface irregularity. The measurement consisted of the following steps.

1. Capturing the images. The histologic sections (Figure 1) were set on an x-ray view box with a ruler for calibration. A digital camera (Coolpix 990, Nikon, Japan) on a stand recorded each section as a JPEG file. All images were calibrated so that the size of a pixel was 9.0 µm.

2. Defining the cartilage contour line. The JPEG files were opened using the image software Photoshop 4.0J (Adobe Systems Inc., San Jose, CA, USA). The articular cartilage surfaces of the tibia and femur were manually delineated pixel by pixel. The rest of the histologic section was erased. The lines representing femoral and tibial articular cartilage contours were saved separately as bitmap files.

3. Analyzing the cartilage contour line. The cartilage contour files were opened with LabVIEW software version 5.0 (National Instruments, Austin, TX, USA). Each pixel of the cartilage contour was numbered from start to end (Figure 1, bottom panel). Every pixel was attributed an X-Y coordinate calculated as the average of 11 consecutive points: the pixel in question and the 5 preceding and 5 following pixels. For example, the X-Y coordinate of point 50 is the average of X-Y coordinates of points 45–55, the X-Y coordinate of point 51 is the average of 46–56, and so on. This operation smoothed the irregularity inherent to digital images. Smoothing is necessary since the relationship between 2 pixels can only be 0 or 45°. Without smoothing, every pair of pixels at a 45° angle to each other would be read as a surface irregularity.

Once the X-Y coordinate of each pixel was determined, we established a reference point. The reference point used for the curved femoral cartilage contour was the mid-distance of a straight line joining the first and last pixel of the cartilage contour line (Figure 2A). The reference point used for flat tibial articular contour was located at a distance "x" from both the first and last pixel. The distance "x" corresponded to the length of a straight line joining the first and last pixel of the tibia contour line (Figure 2B).

Once the reference points were set, the distances between them and each pixel of the contour line were measured (Figure 3A). These distances were plotted on the Y-axis of a graph with the pixel number on the X-axis (Figure 3B). We calculated the first derivative of the distance data, which provided the rate of change in the length between each pixel of the contour line and its reference point. The plot represented the curvature of the articular cartilage surface. We then calculated the second derivative of the distance data, which provided the rate of change in curvature. This plot represented surface irregularities. The second derivative data were converted to absolute values (Figure 4).

4. Calculating the percentage cartilage surface irregularity. Portions of the cartilage contour line that exceeded a threshold of 18.0 µm/pixel^2 were considered irregular. This ratio was arbitrarily defined to obtain an optimal signal-to-noise ratio.

The number of pixels above this threshold divided by the total number of pixels in a cartilage contour line constituted the percentage irregularity of an articular cartilage surface. Histologic artifact or noncartilaginous lesions of the cartilage surface were excluded.

Test-retest reliability and comparison to a gold standard from the materials sciences. The methods to analyze the surface irregularity were entirely automated except for manual delineation of the cartilage contour line. To assess the reproducibility of the method, we randomly remeasured 10 cartilage contour curves. No gold standard exists in biological sciences to compare the results of these methods. In materials sciences, methods exist to determine roughness[36,37]. We collected data with one of these methods to compare with our methods. Briefly, the cartilage contour lines were regarded as having been recorded by an electronic stylus virtually moving on the surface of the joint while sampling the contour line at a rate of 1000 samples per second. To extract the roughness from the contour line, the data were fed through a 2-pass first-order filter with the low pass frequency f_c set at 20 Hz. The result was a filtered or "polished" curve that matched the contour line. Then the roughness curve was obtained by subtracting the filtered data from the contour line. The reasons for a 2-pass filter are to remove time lag and achieve a better fit to the contour line. The pixel size was set at 9 µm and threshold at 7 µm.

Statistical analysis. The software program SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA), was used to build the database and perform the statistical analysis. Surface irregularities were reported separately for femur and tibia, as well as the average of the 2 bones. The effect of the intervention (immobilization, sham-operation, and non-operation) on the percentage of surface irregularity was analyzed statistically using ANOVA with post-hoc Bonferroni correction for the 5 time points (2, 4, 8, 16, and 32 weeks). Corrected p values at $p < 0.05$ were regarded as statistically significant. The effect of duration of immobility on the percentage of femur and tibia surface irregularity was analyzed using a best-fitting curve analysis. The sensitivity and specificity of the methods to detect immobi-

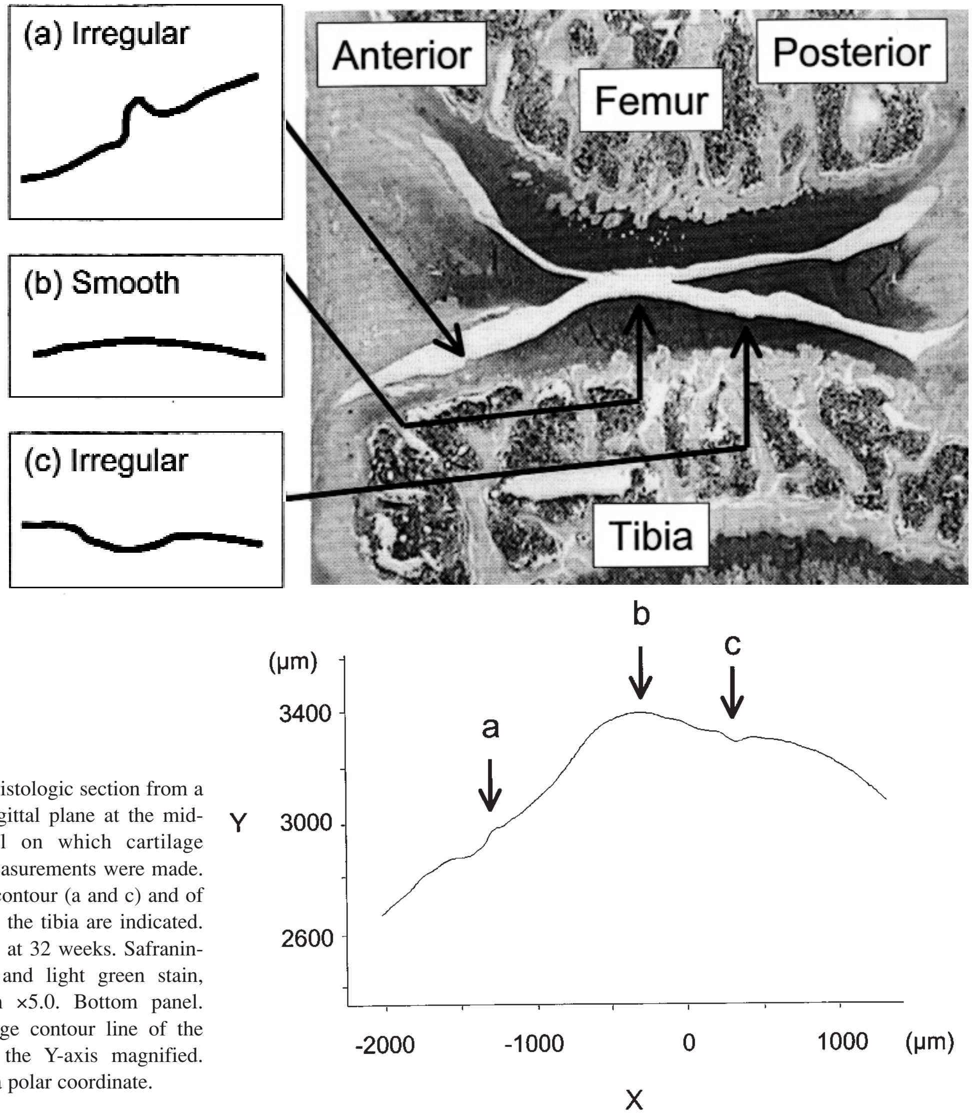

Figure 1. Top panel. Histologic section from a rat knee cut in the sagittal plane at the mid-medial condyle level on which cartilage surface irregularity measurements were made. Stretches of irregular contour (a and c) and of smooth contour (b) on the tibia are indicated. Immobilized specimen at 32 weeks. Safranin-O, iron-hematoxylin, and light green stain, original magnification ×5.0. Bottom panel. Tibial articular cartilage contour line of the same specimen with the Y-axis magnified. Each pixel was given a polar coordinate.

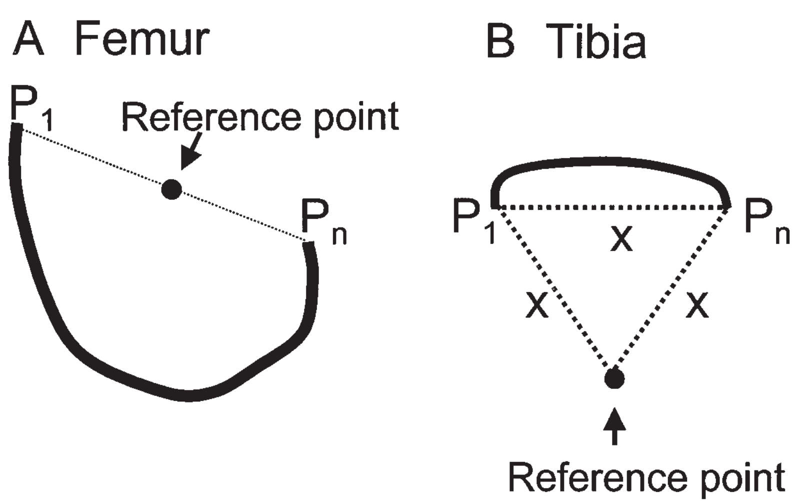

Figure 2. Reference points for femur and tibia were set according to the shape of the cartilage surfaces. A. The reference point for the femur was the midpoint of a straight line connecting the first and last pixel of the contour line (p_1 and p_n). B. The reference point for the tibia was set equidistant from the first and last pixel of the contour line (p_1 and p_n) at a distance "x" corresponding to the length of a straight line connecting first and last pixel of the contour line.

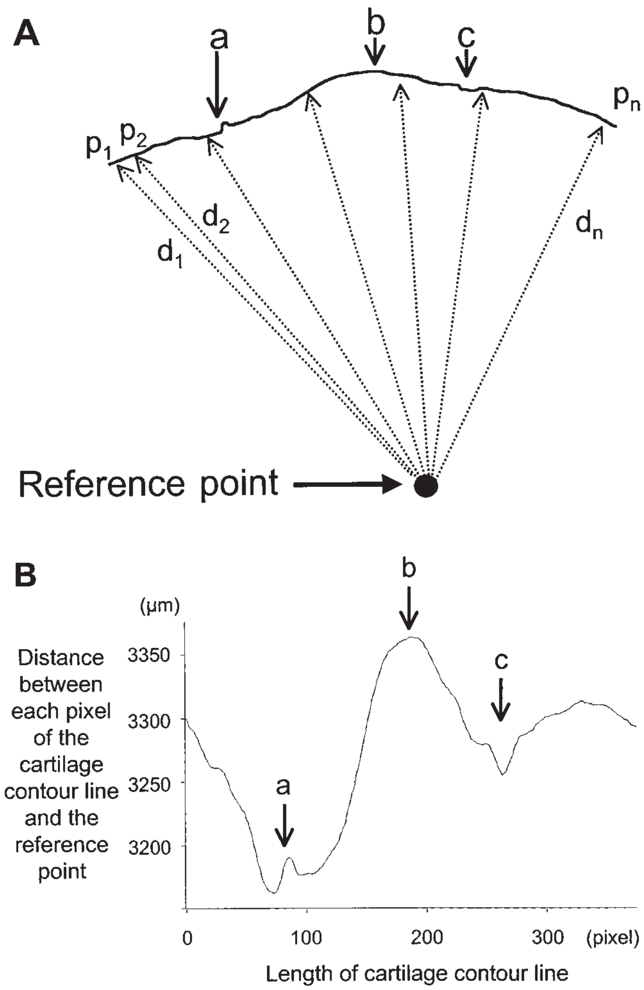

Figure 3. The distance between the reference point and each pixel of a cartilage contour line were measured (panel A) and plotted on a graph (B). The distance fluctuated markedly at the 2 irregular sites (a and c). At the smooth site (b), the distance changed gradually.

lized versus sham-operated or non-operated knees were examined using a 2 × 2 table. To determine the cutoff point, we studied the receiver-operator characteristic (ROC) curve of the data. Test-retest reliability and comparison to a gold standard from materials sciences were analyzed by correlating the first and second readings and expressing the Pearson coefficient.

RESULTS

Twenty-seven knees were excluded due to fracture, technical failure, infection, or processing artifact. One femur datum was also excluded due to major processing artifacts. The method for calculating cartilage surface irregularities was applied successfully to 100 femoral and 101 tibial specimens (Table 1). Non-operated rat femurs displayed 3.2 ± 0.5% of surface irregularity. For non-operated tibias, irregularities covered 3.0 ± 0.8% of the surface. The average of femur and tibia data showed 3.1 ± 0.5% of surface irregularity.

Effect of immobilization: femur. The surface of the femoral

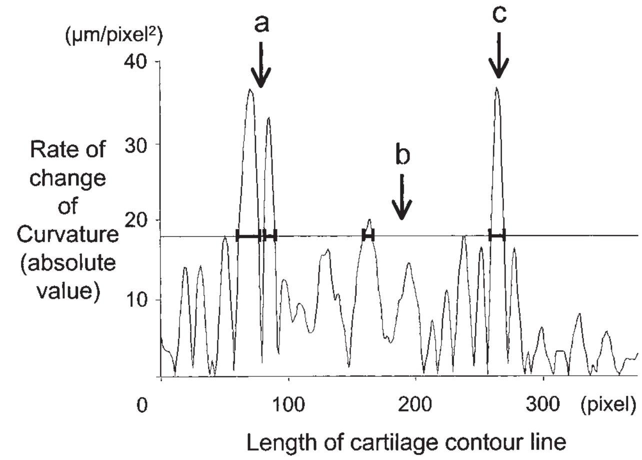

Figure 4. Graph of absolute values of the second derivative of the distance data. The ratio of pixels above the threshold to the total number of pixels of a cartilage contour line defined the percentage of surface irregularity. The sites of irregular contour (a and c) exceeded the threshold. One stretch of cartilage contour other than (a) and (c), which revealed minimal irregularity on histologic section, was slightly over threshold. This represented a limitation in signal-noise discrimination of the methods. The site of smooth contour (b) remained under the threshold.

cartilage of immobilized knees showed a greater percentage of irregularity than that of sham-operated knees 4, 8, 16, and 32 weeks after intervention (Table 1). The immobilized knees showed more irregularity also when compared to non-operated knees at all time points, 2, 4, 8, 16, and 32 weeks after intervention (all $p < 0.05$). Sham-operated knees showed no difference compared with non-operated knees at all time points (Table 1).

Effect of immobilization: tibia. Tibial results were very similar to femoral data. In immobilized knees, the tibial cartilage showed significantly more surface irregularity compared to sham-operated knees 2, 4, 16, and 32 weeks after onset of immobilization (all $p < 0.05$) (Table 1). They also displayed more surface irregularity compared to non-operated knees at 4, 16, and 32 weeks (all $p < 0.05$) (Table 1). The sham-operated knees again showed no difference when compared to non-operated knees at all time points (Table 1).

Effect of immobilization: combined femur and tibia data. The combined data from immobilized femur and tibia revealed, as expected, more irregularity than both the sham-operated and the non-operated knees at all time points (all $p < 0.05$) (Figure 5). Sham-operated knees still showed no difference compared with non-operated knees at all time points (Figure 5).

Effect of duration of immobilization. The "best-fitting curve" describing the effect of duration of immobilization on cartilage surface irregularity on the combined femur and tibia data was: % irregularity = 6.6 + 2.1 ln (t), where t was the duration of immobilization in weeks ($F = 59.3$, p <

Table 1. Surface irregularity of femur and tibia cartilage.

	Non-operated	Operated	2 Weeks	4 Weeks	8 Weeks	16 Weeks	32 Weeks
Femur	3.2 ± 0.5%, n = 11	Immobilized	5.8 ± 0.8%†, n = 14	10.2 ± 1.4%*†, n = 11	16.8 ± 1.8%*†, n = 4	12.2 ± 1.8%*†, n = 10	13.6 ± 1.5%*†, n = 13
		Sham	4.3 ± 0.7%, n = 10	4.0 ± 0.7%, n = 8	6.3 ± 0.2%, n = 2	4.6 ± 0.5%, n = 8	3.9 ± 0.9%, n = 9
Tibia	3.0 ± 0.8%, n = 11	Immobilized	5.1 ± 0.8%*, n = 15	10.8 ± 1.0%*†, n = 11	7.2 ± 1.8%, n = 4	15.2 ± 2.3%*† n = 10	14.1 ± 1.9%*†, n = 13
		Sham	1.8 ± 0.7%, n = 10	4.7 ± 1.3%, n = 8	3.4 ± 0.2%, n = 2	5.1 ± 0.6%, n = 8	2.9 ± 0.5%, n = 9

* Significant differences between immobilized and sham-operated knees, $p < 0.05$. † Significant differences between immobilized and non-operated knees, $p < 0.05$. There was no difference between sham-operated and non-operated knees at any time point.

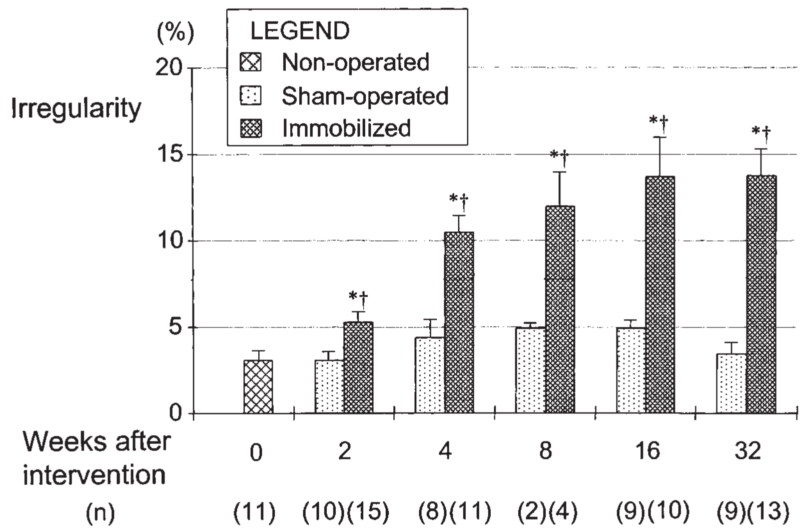

Figure 5. Graph of combined femur and tibia cartilage surface irregularity. Immobilized knees showed more irregularity than sham-operated knees at all time points. Irregularity was observed 2 weeks after intervention and plateaued from 8 to 32 weeks. *Significant differences between immobilized and sham-operated knees, $p < 0.05$. †Significant differences between immobilized and non-operated knees, $p < 0.05$. There was no difference between sham-operated and non-operated knees at any time point. Error bars corresponded to 1 SEM.

0.001). Since a logarithmic curve cannot include a duration of 0 week, the non-operated knees were entered as 0.1 week. The logarithmic relationship showed that surface irregularity increased sharply just after onset of immobilization and plateaued at roughly 8 weeks until 32 weeks.

Sensitivity and specificity of the method. Analysis of the ROC curve determined that a cutoff of 6% conferred the current methods optimal sensitivity/specificity qualities. Therefore, specimens whose surface irregularity exceeded 6.0% were defined as "abnormal," and those below 6.0% were "normal." In the combined femur and tibia data, the methods detected 42 out of 53 immobilized knees as being abnormal, for a sensitivity of 79%. Since 2 weeks of immobilization is a short time to produce morphologic cartilage changes, we analyzed data excluding animals immobilized for only 2 weeks. Thirty-six (36) out of 38 knees were abnormal, increasing the sensitivity of the methods to detect immobilized knees to 95%. Forty-four (44) of 48 sham-operated and non-operated knees had normal surfaces, defining a specificity of this method at 92%.

Test-retest reliability and comparison to a gold standard from the materials sciences. We validated these results by performing test-retest reproducibility on 10 specimens to assess the effect of manual delineation of the cartilage contour, the only nonautomated part of the methods. The results showed a Pearson correlation coefficient of 0.981 between the repeated and the original reading ($p < 0.001$). We compared our results to the materials sciences electronic stylus method. The 201 data points from femur and tibia cartilage were compared. Pearson correlation coefficient was 0.859 ($p < 0.001$).

DISCUSSION

Surface irregularity is one consistent feature of articular cartilage degeneration due to various diseases[12,18,21-27,32,39,40]. Histologic qualitative and semiquantitative methods for evaluating irregularity have been reported[12,18,21-27], but they were not sensitive enough to measure surface irregularity as a criterion in quantitative studies of cartilage degeneration. Newer technologies — atomic force microscopy, confocal microscopy, laser profilometry, and ultrasound — face limitations in their applicability to measure cartilage surface irregularity *in vitro* or *in vivo*, particularly when related to immobilization[33-35]. We designed histology based methods to quantify surface irregularity of articular cartilage and applied them to a rat knee contracture model.

Methods to quantify articular cartilage surface irregularity. We measured the distances between a reference point and each pixel in digitized images of a cartilage contour line and then calculated the absolute value of the second derivative of these distances. Cartilage surface irregularity was defined as a ratio of segments of a contour line above a threshold to the total length of the contour. The methods we developed have the advantages of being quantitative, valid, and fully automated except for the delineation of the cartilage contour lines. They strongly correlated with a gold standard used in materials sciences.

These methods have the potential to follow the progression of cartilage degeneration in various experimental models of arthritis. Importantly, they could be used to precisely measure the effect of an intervention or a treatment (e.g., chondroprotective agent, etc.) on the course of cartilage degeneration in such models. Our methods show high sensitivity and specificity for the detection of pathologic changes in a contracture model.

Recently, magnetic resonance (MR) technology has been used to measure cartilage degeneration[41]. Xia, *et al* evaluated histologic zones of dog shoulder articular cartilage with microscopic MR (resolution 13.7 µm per pixel)[42]. They found a good correlation with measurements obtained by light microscopy[42]. Microscopic MR scanners can provide a resolution of 10 µm per pixel[43], which is comparable to the image resolution we used in this study (9 µm per pixel). Therefore, our methods coupled to microscopic MR images will enable clinical research on cartilage surface irregularity. Such noninvasive, *in vivo* measurement of cartilage surface irregularity may prove to be a key marker of cartilage degeneration clinically.

Raynauld, *et al* used 3D MR to quantify articular cartilage volume and reported a decrease of cartilage volume in OA knees over one year[44]. However, cartilage degeneration does not always involve cartilage volume loss, especially in early stages. Indeed, cartilage degeneration should be detected much earlier. Detection of changes taking months or years to develop is of limited usefulness in a clinical setting. Our methods detected alterations after only 2 weeks of immobilization. An early diagnosis of cartilage degeneration in OA or RA or during immobilization may allow immediate measures to forestall further damage and improve outcome of treatment. The methods we developed may also provide valuable longitudinal data on the natural course of cartilage degeneration in various diseases and provide a unique tool to measure the effect of treatment.

Finally, a classification of cartilage degeneration in MR images used by Yulish, *et al* is based on quantitative cartilage thickness and semiquantitative surface irregularity assessment[45]. This classification can be improved with the methods we present.

One limitation of our methods is the discrimination of signal from noise. This becomes evident looking at the results of normal knees. Theoretically, no irregularity should be present; still, they exhibited an average of 3% surface irregularity. This signal can be modulated by conditions such as resolution of image (pixel size), methods chosen for digital smoothing, position of the reference point, and setting of the threshold. We selected the optimal parameters comparing the signal-to-noise ratio for our specific use, and adjustment for each application is required. Also, programs can be written for automatic delineation of the cartilage contour line.

Surface irregularity of articular cartilage in contractures. The prevalence of cartilage surface irregularities was higher in rat knees developing contractures secondary to immobilization compared with sham-operated knees, confirming our first hypothesis that contracture of a joint induces a surface irregularity.

Helminen, *et al*[23,24] and Jurvelin, *et al*[25] studied rabbit knees immobilized by a splint and classified the cartilage surface alterations on a 6-grade scale: smooth, slightly rough, rough, knobby, leafy/striated, and unclassified. They reported a decrease in smooth surface area and an increase in slightly rough and rough areas compared to controls after 6 to 8 weeks' immobilization. Our findings confirmed their observation, and in addition, quantified the surface irregularities and followed them over longer time points.

Duration of immobilization had a significant and characteristic effect on surface irregularity, which confirmed our second hypothesis. Irregularities were present as early as 2 weeks after immobilization, continued to increase 4 and 8 weeks after intervention, and plateaued thereafter (16 and 32 weeks). Jurvelin, *et al*, using the same 6-grade scale, found that surface irregularity appeared after one week and plateaued after about 8 weeks of immobilization[26]. Hong, *et al*[27] also studied surface irregularity in the rat patella between 6 hours and 6 months of immobilization employing the semiquantitative methods of Jurvelin, *et al*[25]. They observed fewer "smooth" areas and more "rough" areas starting at one week and progressing linearly for up to 6 months with no plateau. The finding of a plateau at 8 weeks in Jurvelin's study and absence of a plateau in Hong's study are contradictory. In both cases, the discriminative limitation of the assessment methods, which combined several qualitative variables and area percentages, may be one reason. The surgical ligation method used by Hong, *et al* may have allowed some motion that perpetuated OA conditions. Cartilage irregularity may also evolve along different patterns according to the animal model and experimental conditions. We found a plateau in the progression of surface irregularity after 8 weeks. Our larger sample size and extended time of observation allowed us to identify a logarithmic rather than a linear relationship between time and surface irregularity. The plateau after 8 weeks can be interpreted as a sign of completion of cartilage adaptation to immobilization.

Why cartilage surface irregularity arises remains unclear. Disturbance in the arrangement and continuity of collagen II fibers in the most superficial cartilage layer has been proposed[21,46]. Józsa, *et al* reported that empty pits on the cartilage surface secondary to chondrocyte death might be followed by a collapse of the superficial layer[47]. The type of immobilization may affect the appearance of surface irregularity, e.g., increased pressure causing cartilage necrosis[18,48]. Studies comparing surface irregularity at points of contact between 2 bones of a joint and away from these points of contact would clarify the effect of increased pressure on the development of cartilage surface irregularity.

We developed methods to measure articular cartilage surface irregularity and applied them to a rat knee contracture model. Cartilage surface irregularity appeared after 2 weeks of immobilization and progressed rapidly to plateau after 8 weeks. The methods we presented can evaluate cartilage damage, progress of disease, and response to treatment in a research setting. Combined with high resolution MR imaging, these methods may find clinical applications for early detection of and immediate action on cartilage degeneration.

ACKNOWLEDGMENT

We thank Clare Booth for her technical assistance, Dorothyann Curran for assisting with data analysis, Shawn Miller for help in the computer program, and Dr. David Jackson for editing the manuscript.

REFERENCES

1. Palmoski M, Perricone E, Brandt KD. Development and reversal of a proteoglycan aggregation defect in normal canine knee cartilage after immobilization. Arthritis Rheum 1979;22:508-17.
2. Palmoski MJ, Brandt KD. Running inhibits the reversal of atrophic changes in canine knee cartilage after removal of a leg cast. Arthritis Rheum 1981;24:1329-37.
3. Tammi M, Saamanen A-M, Jauhiainen A, Malminen O, Kiviranta I, Helminen H. Proteoglycan alterations in rabbit knee articular cartilage following physical exercise and immobilization. Connect Tissue Res 1983;11:45-55.
4. Behrens F, Kraft EL, Oegema TR. Biochemical changes in articular cartilage after joint immobilization by casting or external fixation. J Orthop Res 1989;7:335-43.
5. Videman T, Michelsson JE, Rauhamaki R, Langenskiold A. Changes in ^{35}S-sulphate uptake in different tissues in the knee and hip regions of rabbits during immobilization, remobilization and the development of osteoarthritis. Acta Orthop Scand 1976;47:290-8.
6. Eronen I, Videman T, Friman C, Michelsson JE. Glycosaminoglycan metabolism in experimental osteoarthrosis caused by immobilization. Acta Orthop Scand 1978;49:329-34.
7. van Kampen GPJ, van de Stadt RJ. Cartilage and chondrocyte responses to mechanical loading in vitro. In: Helminen HJ, Kiviranta I, Tammi M, Saamanen A-M, Paukkonen K, Jurvein J, editors. Joint loading. Bristol, England: Wright; 1987:112-25.
8. Jortikka MO, Inkinen RI, Tammi MI, et al. Immobilisation causes longlasting matrix changes both in the immobilised and contralateral joint cartilage. Ann Rheum Dis 1997;56:255-61.
9. Paukkonen K, Jurvelin J, Helminen HJ. Effects of immobilization on the articular cartilage in young rabbits. A quantitative light microscopic stereological study. Clin Orthop 1986;206:270-80.
10. Kiviranta I, Jurvelin J, Tammi M, Saamanen A-M, Helminen HJ. Weight bearing controls glycosaminoglycan concentration and articular cartilage thickness in the knee joints of young beagle dogs. Arthritis Rheum 1987;30:801-9.
11. Saamanen A-M, Tammi M, Kiviranta I, Jurvelin J, Helminen HJ. Maturation of proteoglycan matrix in articular cartilage under increased and decreased joint loading. A study in young rabbits. Connect Tissue Res 1987;16:163-75.
12. Thaxter TH, Mann RA, Anderson CE. Degeneration of immobilized knee joints in rats. Histological and autoradiographic study. J Bone Joint Surg Am 1965;47:567-85.
13. Muller FJ, Setton LA, Manicourt DH, Mow VC, Howell DS, Pita JC. Centrifugal and biochemical comparison of proteoglycan aggregates from articular cartilage in experimental joint disease and joint instability. J Orthop Res 1994;12:498-508.
14. Videman T, Eronen I, Candolin T. [^{3}H]proline incorporation and hydroxyproline concentration in articular cartilage during the development of osteoarthritis caused by immobilization. A study in vivo with rabbits. Biochem J 1981;200:435-40.
15. Tammi M, Kiviranta I, Peltonen L, Jurvelin J, Helminen HJ. Effects of joint loading on articular cartilage collagen metabolism: assay of procollagen prolyl 4-hydroxylase and galactosylhydroxylysyl glucosyltransferase. Connect Tissue Res 1988;17:199-206.
16. O'Connor KM. Unweighting accelerates tidemark advancement in articular cartilage at the knee joint of rats. J Bone Miner Res 1997;12:580-9.
17. Haapala J, Arokoski JPA, Hyttinen MM, et al. Remobilization does not fully restore immobilization induced articular cartilage atrophy. Clin Orthop 1999;362:218-29.
18. Evans EB, Eggers GWN, Bulter JK, Blumel J. Experimental immobilization and remobilization of rat knee joints. J Bone Joint Surg Am 1960;42:737-58.
19. Sood SC. A study of the effects of experimental immobilisation on rabbit articular cartilage. J Anat 1971;108:497-507.
20. Paukkonen K, Helminen HJ, Tammi M, Jurvelin J, Kiviranta I, Saamanen A-M. Quantitative morphological and biochemical investigations on the effects of physical exercise and immobilization on the articular cartilage of young rabbits. Acta Biol Hung 1984;35:293-304.
21. Finsterbush A, Friedman B. Early changes in immobilized rabbits knee joints: a light and electron microscopic study. Clin Orthop 1973;92:305-19.
22. Candolin T, Videman T. Surface changes in the articular cartilage of rabbit knee during immobilization. A scanning electron microscopic study of experimental osteoarthritis. Acta Pathol Microbiol Scand [A] 1980;88:291-7.
23. Helminen HJ, Jurvelin J, Kuusela T, Heikkila R, Kiviranta I, Tammi M. Semiquantitative analysis of rabbit knee articular surfaces based on stereomicroscopic examination of the cartilage. Acta Anat

(Basel) 1983;115:319-26.
24. Helminen HJ, Jurvelin J, Kuusela T, Heikkila R, Kiviranta I, Tammi M. Effects of immobilization for 6 weeks on rabbit knee articular surfaces as assessed by the semiquantitative stereomicroscopic method. Acta Anat (Basel) 1983;115:327-35.
25. Jurvelin J, Kuusela T, Heikkila R, Pelttari A, Kiviranta I, Tammi M. Investigation of articular cartilage surface morphology with a semiquantitative scanning electron microscopic method. Acta Anat (Basel) 1983;116:302-11.
26. Jurvelin J, Helminen HJ, Lauritsalo S, et al. Influence of joint immobilization and running exercise on articular cartilage surfaces of young rabbits. A semiquantitative stereomicroscopic and scanning electron microscopic study. Acta Anat (Basel) 1985;122:62-8.
27. Hong SP, Henderson CNR. Articular cartilage surface changes following immobilization of the rat knee joint. A semiquantitative scanning electron-microscopic study. Acta Anat (Basel) 1996;157:27-40.
28. Meachim G, Ghadially FN, Collins DH. Regressive changes in the superficial layer of human articular cartilage. Ann Rheum Dis 1965;24:23-30.
29. Meachim G. Light microscopy of Indian ink preparation of fibrillated cartilage. Ann Rheum Dis 1972;31:457-64.
30. Bullough P. The significance of the fine structure of articular cartilage. J Bone Joint Surg Br 1968;50:852-7.
31. Walker PS, Sikorski J, Dowson D, Longfield MD, Wright V, Buckley T. Behaviour of synovial fluid on surfaces of articular cartilage. A scanning electron microscope study. Ann Rheum Dis 1969;28:1-14.
32. Redler I, Zimny ML. Scanning electron microscopy of normal and abnormal articular cartilage and synovium. J Bone Joint Surg Am 1970;52:1395-404.
33. Adler RS, Dedrick DK, Laing TJ, et al. Quantitative assessment of cartilage surface roughness in osteoarthritis using high frequency ultrasound. Ultrasound Med Biol 1992;18:51-8.
34. Chiang EH, Laing TJ, Meyer CR, Boes JL, Rubin JM, Adler RS. Ultrasonic characterization of in vitro osteoarthritic articular cartilage with validation by confocal microscopy. Ultrasound Med Biol 1997;23:205-13.
35. Hu K, Radhakrishnan P, Patel RV, Mao JJ. Regional structural and viscoelastic properties of fibrocartilage upon dynamic nanoindentation of the articular condyle. J Struct Biol 2001;136:46-52.
36. International Organization for Standardization. Determination of roughness parameters Ra, Rz, Rmax by means of stylus instruments; terms, measuring conditions (DIN 4768); 1990. [cited April 11, 2003]. Available from: http://www.taylor-hobson.com/latestiso.htm
37. International Organization for Standardization. Profile filters for electrical contact stylus instruments application; phase-corrected filters (DIN 4777); 1990. [cited April 11, 2003]. Available from: http://www.taylor-hobson.com/latestiso.htm
38. Trudel G, Uhthoff HK. Contractures secondary to immobility: Is the restriction articular or muscular? An experimental longitudinal study in the rat knee. Arch Phys Med Rehabil 2000;81:6-13.
39. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surg Am 1971;53:523-37.
40. Clark JM, Simonian PT. Scanning electron microscopy of "fibrillated" and "malacic" human articular cartilage: technical considerations. Microsc Res Tech 1997;37:299-313.
41. Calvo E, Palacios I, Delgado E, et al. High-resolution MRI detects cartilage swelling at early stage of experimental osteoarthritis. Osteoarthritis Cartilage 2001;9:463-72.
42. Xia Y, Moody JB, Burton-Wurster N, Lust G. Quantitative in situ correlation between microscopic MRI and polarized light microscopy studies of articular cartilage. Osteoarthritis Cartilage 2001;9:393-406.
43. Xia Y. Contrast in NMR imaging and microscopy. Concepts Magn Res 1996:8:205-25.
44. Raynauld JP, Kauffman C, Godbout B, et al. Knee osteoarthritis progression evaluated by magnetic resonance imaging and novel quantification software tool [abstract]. Arthritis Rheum 2000;43 Suppl:399.
45. Yulish BS, Montanez J, Goodfellow DB, Bryan PJ, Mulopulos GP, Modic MT. Chondromalacia patellae: assessment with MR imaging. Radiology 1987;164:763-6.
46. Gardner DL. The nature and causes of osteoarthrosis. BMJ 1983;286:418-24.
47. Jozsa L, Jarvinen M, Kannus P, Reffy A. Fine structural changes in the articular cartilage of the rat's knee following short-term immobilisation in various positions: A scanning electron microscopical study. Int Orthop 1987;11:129-33.
48. Torzilli PA, Grigiene R, Borrelli J Jr, Helfet DL. Effect of impact load on articular cartilage: cell metabolism and viability, and matrix water content. J Biomech Eng 1999;121:433-41.