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Surface topography of viable articular cartilage measured with scanning white light interferometry

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Summary

Objective: By means of scanning white light interferometry, develop a noncontact, nondestructive technique capable of measuring surface topography of viable cartilage.

Methods: Using full thickness cylindrical cartilage explants obtained from bovine calf knees, experiments were performed to produce a surface preparation protocol that yields highly repeatable topographical measurements while maintaining cartilage viability. To further validate the technique, a series of human talar cartilage samples, displaying varying degrees of cartilage degeneration, was then subjected to interferometric measurements and compared to their histology.

Results: A key aspect of the technique of surface topographic measurement by interferometry was the development of an optimal surface preparation process. The technique was successfully validated against standard 2-D profilometry. The intrinsic variability of the technique is less than 2%, which is much less than the average point-to-point variability of 17% observed across a cartilage specimen. The technique was hence sufficiently sensitive to readily detect differences in roughness between surfaces of healthy cartilage in different locations on the bovine knee. Thus, the average roughness of the medial explants exceeded that of the lateral explants by 0.35 μm Ra ($P = 0.003$) and the roughness of the trochlear explants exceeded that of the condylar explants by 0.55 μm Ra ($P < 0.0001$). Also, applying this technique to diseased human talar cartilage samples, a statistically significant increase in the average surface roughness value per unit increase in histological degeneration score was observed ($\geq$0.2 μm Ra, $P \leq 0.041$), making surface roughness obtained *via* interferometry a useful parameter for evaluating cartilage health nondestructively.

Conclusions: The aim of developing a protocol based on white light interferometry to measure the surface topography of viable articular cartilage was achieved. This interferometric technique opens the door to monitoring the surface topography of live cartilage, as is desirable for *ex vivo* tests on cartilage explants.

Key words: Bovine cartilage, Human talar cartilage, White light interferometry, Topography.

Introduction

The surface of articular cartilage has been analyzed for topography[1], composition[2], fine structure[3], collagen orientation[4] and features such as asperities and grooves[5]. Its distinct topography allows for transport of nutritious fluid under elastohydrodynamic lubrication conditions[6], and changes in its topography impact lubrication mechanisms[7]. Topographical measurements have been used to evaluate the progression of cartilage wear[8] and are a prerequisite for *ex vivo* explant cultures, which have become increasingly popular in the field of mechanobiology. In particular, *ex vivo* wear tests designed to evaluate artificial cartilage repair materials intended to articulate against natural cartilage are currently being established[9–11].

Different techniques have been used to investigate the topography of articular cartilage including photography[1], scanning electron microscopy (SEM)[2], stylus profilometry[7], stereo photogrammetry[12], magnetic resonance imaging[13], atomic force microscopy (AFM)[14], and scanning white light interferometry[15]. Among these techniques, only AFM, profilometry, and interferometry provide the quantitative topographical data necessary to understand the tribological behavior of cartilage surface. Although used extensively, SEM does not provide quantitative measurements of the surface topography and is used primarily to study the arrangement of extracellular matrix components within the tissue[16].

Although AFM has been used to measure the surface roughness of cartilage at the submicron level[14], its strength is in evaluating functional and mechanical properties of individual matrix components[17], as well as the frictional properties of cartilage at submicron level[18]. A limitation of AFM is the small size of the areas ($\sim$100 μm^2) that can be scanned, making it impractical for measuring surface roughness over macroscopic areas ($\geq$25,000 μm^2), as required for example in the evaluation of cartilage wear[11].

Interferometry has been used to evaluate cell adhesion on composite films in cell cultures[19] and to measure the surface profile and roughness of thick biomimetic hydroxyapatite layers[20] and of substrate of cell templates[21]. Devices with modified interferometric techniques, such as optical coherence tomography, have been used to perform 3-D volumetric reconstruction of biological tissues such as the

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cornea, lens and retina[22,23]. The use of interferometry to characterize the surface topography of cartilage appears very limited to date. In a recent study[15], the technique was utilized to examine features such as pits and "humps", and to quantify the topography for parameters such as roughness (R_a), root-mean-square deviation (R_q), and skew (R_{sk}). However, the procedure used to prepare the cartilage surface for topographical measurements was not reported. In later studies, while examining larger surface areas, interferometry was deemed less appropriate due to tissue dehydration during measurement and was thus replaced with stylus profilometry[10]. Stylus profilometry, originally used in the 1960s to establish that surface topography is a valuable indicator of cartilage health[7], remains the dominant technique for determining cartilage topography[10]. Typically used in single trace mode, the profilometer captures a 2-D linear profile of the surface. Multiple parallel traces are required for 3-D data. Contact-based and slow, stylus profilometry can mar the cartilage surface and reduce tissue viability. Profilometry is therefore inadequate for *ex vivo* cartilage wear tests where nondestructive monitoring of changes in surface topography is crucial to an understanding of the underlying damage mechanisms[10,11]. In view of these limitations with profilometry and the current availability of sophisticated interferometry systems capable of making topography measurements in seconds, reconsideration of the interferometry technique becomes attractive.

Our aim was therefore to develop a noncontact, nondestructive technique for measuring surface topography of viable cartilage explants in a fast, accurate, and reliable manner using a commercially available scanning white light interferometry system. We also demonstrated the utility of the technique in two applications, namely, for quantifying topography as a function of location on the bovine knee and to determine the correlation of different grades of cartilage degeneration to surface roughness values.

Materials and methods

The interferometric technique entails two major steps: proper preparation of the cartilage surface and topographical measurement of the prepared region. To address the former, experiments were performed to optimize the surface preparation parameters and produce a surface preparation protocol that yields highly repeatable topographical measurements while maintaining cartilage viability. Standard stylus profilometry was then used to effect a verification of the optimized interferometry technique. The technique was subsequently demonstrated in two applications, as mentioned in the aim of this study. A flowchart summarizing this development process is shown in Fig. 1.

SPECIMEN PREPARATION

Unless otherwise stated, all the specimens were full thickness cartilage explants, 14 mm in diameter, obtained from 6 to 8 month old bovine calf knees from the trochlear and condylar regions. The knees were acquired from a local slaughterhouse (Chiappetti Lamb & Veal Corporation, Chicago, IL) and were from animals slaughtered that same day. During explantation, the cartilage surface was kept moist with Dulbecco's phosphate buffered saline 1X solution. Upon retrieval, all explants were immediately washed and cultured in Dulbecco's modified eagle's medium, type F12 (DMEM-F12) with 10% fetal bovine serum (FBS) at 37°C for 15 to 48 h, except for the explants used in the optimization of the surface preparation method. These explants, for which chondrocyte viability was determined, were cultured for a period of 5 days to equilibrate and stabilize their biosynthetic activity levels[24].

INTERFEROMETRIC SURFACE ROUGHNESS MEASUREMENTS

A state-of-the-art scanning white light interferometry microscope (NewView 6300; Zygo Corp., Middlefield, CT) was used for noncontact surface topography measurements of the cartilage explants. The microscope vertically scans the test area to generate a digital 3-D topographical map of the surface with a vertical resolution of up to 0.1 nm, a value well below the surface roughness values encountered in this work. The cartilage surfaces were measured using a 20 × Mirau interferometric objective with a working distance of 4.3 mm and a lateral resolution of 0.87 μm. The field of view of 0.702×0.527 mm^2 was mapped onto a charge-coupled device (CCD) array of 640 by 480 pixels, yielding up to 307,200 data points per measurement with a spatial sampling of 0.55 μm. Stable interferograms were obtained using the surface preparation developed in this study, indicating the spatial stability of the live tissue and the absence of a discrete fluid film during the measurement. The areal surface roughness, expressed as the arithmetic average R_a, was computed after correcting for geometry, typically a cylinder or a plane. The topography data was otherwise unfiltered.

OPTIMIZATION OF THE SURFACE PREPARATION METHOD

Given the importance of timely wetting of the cartilage surface for maintaining tissue viability[25], experiments were performed to minimize surface preparation time while avoiding the presence of discrete fluid films during the interferometry measurements. These optimization experiments were conducted on trochlear and condylar cartilage explants obtained from eight bovine calf knees. Using a quadratic response surface design of experiment[26], the following surface preparation parameters (input variables) were evaluated multifactorially: whether to blot or not the surface with wipes (Kimwipes®, Product Code 34155; Kimberly-Clark Corp., Dallas, TX), the duration of the applied jet of nitrogen (0, 5 and 10 s) and air-drying time (0, 2.5, and 5 min). Given all the possible combinations of these levels for the input variables and five-fold replication of the mid points (nitrogen 5 s, air-drying 2.5 min), the experiment entailed testing 26 explants. All the topographical measurements were performed at room temperature, 20 ± 3°C. The response variables were total chondrocyte viability, superficial zone viability, and proportion of valid interferograms. The values of these response variables were maximized with respect to the input variables using the optimization scheme of Myers and Montgomery[27]. In this scheme, each input and response variable is assigned a desirability index whose value varies from 0 to 1, where 1 denotes complete fulfillment of the criterion for that parameter. A global optimization parameter, proportional to the geometric mean of all the desirability indices, was then calculated to yield an optimization

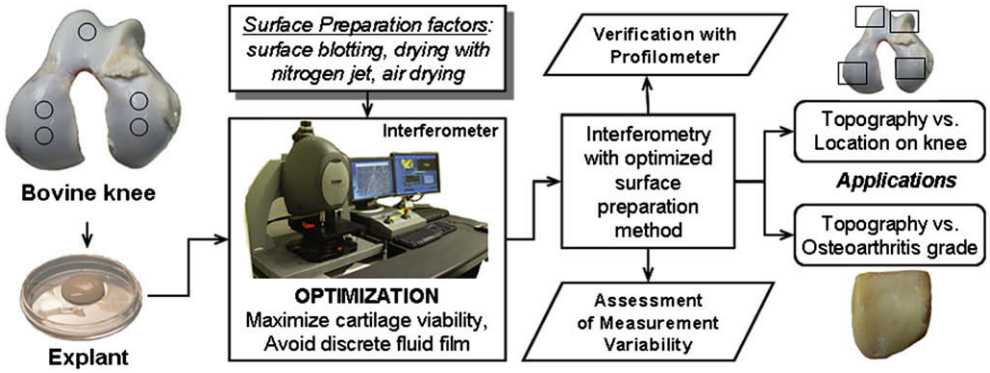

Fig. 1. Flowchart for the experiments and analyses undertaken in this study to develop the interferometric technique for quantifying cartilage surface topography.

surface as a function of the input parameters. The optimized surface preparation protocol used input values in the region that maximized the global optimization parameter. The validity of the chosen values for the input parameters with respect to chondrocyte viability (see next heading) and percentage of valid interferograms was subsequently verified on a set of 30 explants: 15 controls and 15 explants subjected to the chosen surface preparation protocol followed by the interferometric measurement. The cell viability measurements were performed 1 and 7 days after the interferometric measurements. Between measurements, the explants were cultured in DMEM-F12 media with 10% FBS at 37°C.

CHONDROCYTE VIABILITY DETERMINATION

After topographical measurements, full width, 1 mm thick sections were obtained from the condylar and trochlear explants, in the anterior – posterior and in the inferior – superior directions, respectively. The sections were stained in Dulbecco's phosphate buffered saline 1X solution containing calcein AM and ethidium homodimer-1 (LIVE/DEAD Viability/Cytotoxicity Kit; Molecular Probes, OR) and incubated at 37°C for a period of 20 min. The stained sections were imaged using a fluorescence-light microscope (Eclipse TE2000-S; Nikon Instruments Inc, NY), a 5× objective and a CCD camera (SPOT RT-KE Color 3-Shot, Model 7.3×; Diagnostic Instruments, MI). ImageJ software[28] was used to perform live and dead cell counts for the obtained images to quantify total explant cell viability and the cell viability of the superficial zone. The top 15% of the cartilage thickness was defined as representative of the superficial zone of the explants[29]. This method did not distinguish between necrotic and apoptotic cell death.

VERIFICATION OF THE INTERFEROMETRIC MEASUREMENTS

Interferometric and profilometric methods were compared on a set of 16 cartilage explants retrieved from the bovine knee joint of a 6-month old animal. To obtain the maximum range of surface roughness values, explants were retrieved in equal number from four distinct regions on the distal femur, namely, the medial and lateral aspects of the trochlear groove and the medial and lateral condyles. The specimens were stored in culture media at 4°C until use. The surface roughness measurements obtained by interferometry were evaluated against those acquired using a standard stylus profilometer (Mahr Feinpruf S8P Perthometer; Mahr-Perthen, Göttingen, Germany). The developed surface preparation technique was implemented, and the explants were individually measured with the interferometry microscope at four predefined points over the prepared cartilage surface. Four such measurements per cartilage explant were made, located at the corners of a square 5 mm on a side and centered on the disk. The explants were then re-immersed in culture media to maintain hydration, cultured overnight, and identically re-prepared the following day and re-measured on the profilometer. The classical 2-D profilometry measurements were performed using a diamond stylus with a 10 μm tip radius. Profilometry data was acquired along two longitudinal and two transverse linear paths, each pair approximately 2 mm apart and symmetrically positioned about the disk center. Each path yielded eleven consecutive 0.702 mm traces consisting of 708 *x-y* data points with a spatial sampling of 0.99 μm. Each trace was corrected for inclination, and the R_a was calculated from the otherwise unfiltered data, to match the processing for the interferometric measurements. In this manner, a total of 44 R_a measurements per cartilage explant were obtained.

VARIABILITY IN MEASUREMENTS

The *intra-plug* variability (resulting from inherent variation in cartilage surface topography) was obtained for four explants that were subjected to the developed surface preparation method and measured at nine locations on each explant surface, on a 3 × 3 grid with a 2 mm gap between measured areas. The variability was computed as the root-mean-square of the standard deviations for the explants. To determine the *intrinsic* variability (limitation of the microscope), the explants were re-hydrated for 1 h, identically re-prepared, and each consecutively measured 15 times at a single location, with an interval of 15 s between measurements. This location overlapped the central point of the 3 × 3 grid. The intrinsic variability was computed as the standard deviation of these measurements.

LOCATION DEPENDENCE OF SURFACE ROUGHNESS IN LIVE CARTILAGE

Sixteen full thickness cartilage explants, procured from two bovine calf knees, were evenly divided into four groups based on location (trochlear groove, condylar region) and aspect (medial, lateral). They underwent surface roughness measurements by interferometry using the optimized protocol developed in this study. The surface roughness values were evaluated for the effect of cartilage location and aspect by two-way Analysis of variance (ANOVA).

INFLUENCE OF CARTILAGE DEGENERATION ON SURFACE TOPOGRAPHY

Three human ankles with cartilage degeneration characteristic of that seen in osteoarthritis were obtained from two donors aged 66 and 77 years through the Gift of Hope Organ and Tissue Donor Network with IRB (Institutional Review Board) approval from Rush University Medical Center. The joints were thawed from −80°C and stored at 4°C. From each talus, four full thickness, 8 mm in diameter cartilage explants, were procured from representative areas having different visual cartilage degeneration scores[30]. To ensure that the region subsequently sectioned for histology corresponded with the region measured for topography, each explant surface was marked with reference points using a needle dipped in India ink. After surface preparation, the topography was measured at three adjacent areas centered about the plug axis and aligned in the physiological medial-lateral direction. Following the measurements, the explants were prepared for histological examination by fixation, dehydration, paraffin embedding and staining with Safranin O/Fast green. Photomicrographs were then taken with a confocal microscope (Eclipse E600; Nikon Instruments Inc.) at 25× magnification. The 36 areas, each approximately 0.7 mm wide, that matched the topographically measured areas were then cropped from these images. Each image was then blindly graded by an experienced senior investigator (C. Muehleman) using a macroscopic scale from 0 (normal), 1 (shallow fibrillation) to 2 (deep fibrillation)[34].

STATISTICS

The correlation between interferometric and stylus profilometric roughness values was performed with standard regression analysis using the statistical software SPSS® 10.0 (SPSS Inc., Chicago, IL). Cell viability differences between groups and differences between histological cartilage degeneration grades and R_a values were investigated with one-way ANOVA using Design Expert, version 6.0 (Stat-Ease, Inc., Minneapolis, MN). The multifactorial optimization experiments were designed and analyzed by ANOVA using Design Expert. Significance was set to $P < 0.05$. Unless otherwise stated, the ± values following means are standard deviations.

Results

SURFACE PREPARATION PROTOCOL AND CHONDROCYTE VIABILITY

Surface topographic measurement of cartilage by interferometry required the development of an optimal surface preparation process. Optimization experiments to maximize chondrocyte viability and the proportion of valid

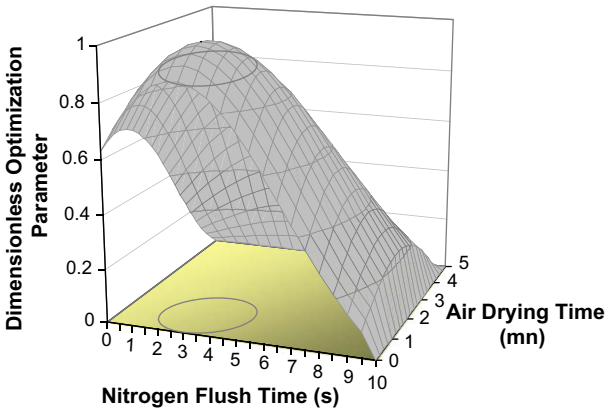

Fig. 2. Optimization surface for the surface preparation and interferometric measurement of the cartilage explants with respect to nitrogen spray and air-drying times. Chondrocyte viability and the fraction of valid interferograms increases with the global optimization parameter. The latter is 0.9 to 1 in the region enclosed by the circle.

Table I
Chondrocyte viabilities in explants that underwent the surface preparation protocol and interferometric topography measurements (Tested explants) vs in explants that did not (Controls). Cell viability was measured 1 and 7 days after the interferometric measurements

	Total explants				Superficial zone			
	Controls 1 day	Tested explants 1 day	Controls 7 days	Tested explants 7 days	Controls 1 day	Tested explants 1 day	Controls 7 days	Tested explants 7 days
Average (% live)	66.0	68.4	67.8	65.9	71.0	71.7	72.0	77.9
Standard deviation (% live)	8.4	7.1	7.2	8.3	15.0	7.3	17.7	10.0
Standard error (% live)	2.2	1.9	1.9	2.2	4.3	1.9	4.7	2.7
Decrease* (% live)		−2.4		1.9		−0.7		−5.9
P value†		0.79		0.26		0.56		0.86
Detectable decrease‡ (% live)		8		8		13		15

*Chondrocyte viability in tested explants minus viability in the controls.

†One-tailed *t*-test to determine if the tested explants have lower cell viability than the controls. The one-tailed test is more severe than the two-tailed test for this purpose.

‡For power = 0.8 and $P = 0.05$.

interferograms, while minimizing nitrogen and air-drying times led to the surface preparation optimization surface shown in Fig. 2, in which a global optimization parameter is plotted as a function of the two preparation inputs. Based on this optimization surface, the following surface preparation method was established: the cartilage surface is lightly blotted with low-lint wipes, sprayed with a jet of nitrogen for 2 to 3 s, using a 1.5 mm diameter nozzle and gas pressure of 40 psi, and air-dried for a period of 1 to 2 min at room temperature. Blotting with wipes was found necessary to maintain cartilage viability because it decreased the time required to remove excess surface fluid compared to just spraying with nitrogen and air-drying. Cartilage explants subjected to the prescribed surface preparation method and interferometric measurement exhibited total and superficial zone chondrocyte viabilities that were not statistically significantly lower than those of controls not subjected to such treatment, as determined 1 and 7 days after the interferometric measurement (Table I). The average cell viability decrease did not exceed 1.9% live cells compared to the controls. The proportion of valid interferograms obtained on this set of explants was 94.6%, excluding one explant with a surface too concave for a proper interferometric measurement.

VERIFICATION OF THE INTERFEROMETRIC MEASUREMENTS

The R_a surface roughness values obtained with this interferometric technique on a set of 16 bovine knee femoral cartilage explants correlate linearly with those obtained by standard stylus profilometry. The slope of the least squares fit line is 0.96, with $R^2 = 0.72$ ($P = 0.0001$) (Fig. 3). The slope is not statistically significant from 1 ($P = 0.81$). Slight remnants of the profilometry traces were detected by interferometry on one specimen, appearing as linear tracks with a depth of ~ 0.3 μm.

VARIABILITY IN THE MEASUREMENTS

The relative standard deviation or variability within a specimen (intra-plug variability) was an average of 17%, whereas the relative standard deviation for repeated measurements on the same spot (intrinsic variability) was <2%. The roughness values were not significantly time-dependent over the 210 s time intervals of these variability measurements. Thus, the last three values of the fifteen values taken for each specimen were not statistically significantly different from the first three values ($P = 0.25$, paired *t*-test).

LOCATION DEPENDENCE OF SURFACE ROUGHNESS IN LIVE CARTILAGE

As an illustration of the application and capability of the interferometric technique, the surface roughness values of cartilage explants procured from different locations on the bovine distal femur were compared. The average R_a of 1.05 ± 0.5 μm for medial explants was significantly higher than the corresponding value of 0.70 ± 0.2 μm for lateral explants ($P = 0.003$). Similarly, the average R_a value of 1.15 ± 0.4 μm for trochlear explants was significantly higher than the value of 0.60 ± 0.1 μm for condylar explants ($P < 0.0001$), although this difference is more prominent on the medial side ($P = 0.0023$ for the interaction term). Differences in topography between locations are also evident in the 3-D spatial plots, with the condylar explants exhibiting more fine structure than the trochlear

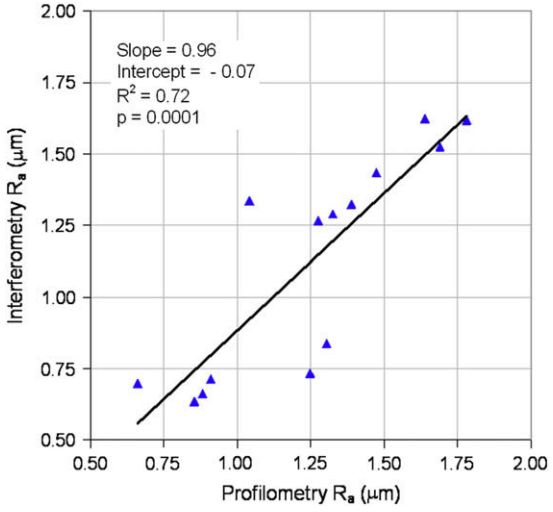

Fig. 3. Correlation between surface roughness measurements made by interferometry and profilometry.

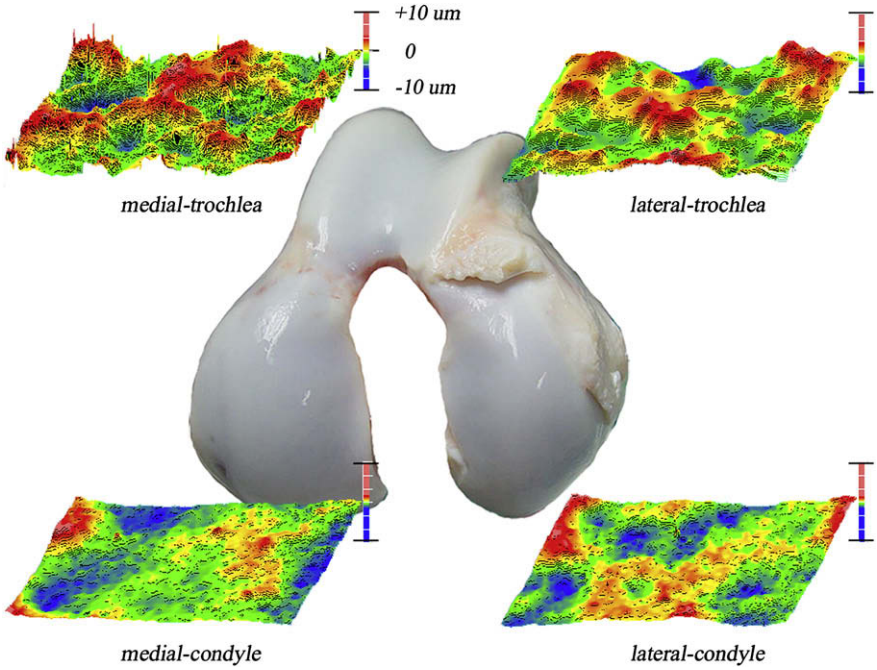

Fig. 4. 3-D plots of the cartilage surface topography at four locations on the bovine knee.

explants, even though the latter have a higher average R_a value (Fig. 4). To the naked eye, the cartilage surfaces appeared smooth with slight "orange peel" texture, and their differentiation based on location and aspect was not evident.

INFLUENCE OF CARTILAGE DEGENERATION ON SURFACE TOPOGRAPHY

In view of their potential clinical relevance, surface roughness measurements were also made on human osteoarthritic cartilage explants to investigate their correspondence to histological cartilage degeneration grades. Using human talar cartilage, the average surface roughness values for grades 0, 1 and 2 were 0.80 ± 0.3 μm, 1.0 ± 0.3 μm and 1.70 ± 0.9 μm, respectively. The differences in surface roughness values between the three grades were all pairwise statistically significant ($P < 0.05$, Fig. 5). Representative histological sections of the talar cartilage used in the study are shown in Fig. 5 for the three grades. Consistent with grade assignment[30,34], there is an increase in chondrocyte death and a decrease in matrix staining with an increase in grade. Surface discontinuities can be seen for grades 1 and 2.

Discussion

The aim of developing an interferometry protocol to measure the surface topography of viable articular cartilage was achieved. The measured R_a values were in good agreement with those obtained with standard stylus profilometry, confirming the accuracy of the interferometric technique. The total time required by interferometry (3–5 min), including surface preparation, was much lower than that required by profilometry (30–35 min) for measuring a similar area, in part because of the imaging capability of the NewView 6300 microscope. Indeed, 3-D topography data is acquired with this microscope, rather than the 2-D profiles, allowing for a more complete and realistic topographical characterization of the surface. Although not inherent to the interferometry method and beyond the scope of this study, the powerful and versatile MetroPro software (Zygo Corp.) controlling the microscope permits a sophisticated analysis of the topography data and the calculation of a wide array of surface roughness parameters, such as the mean peak-to-valley height (R_z), bearing ratio (R_k), flatness, skew (S_k), number of peaks, and number of valleys.

Equally important, the interferometric technique did not significantly decrease the proportion of live cells in the cartilage when compared to hydrated controls. Being noncontact, the technique does not leave marks or introduce artifacts on the surface. Also, the entire process of surface preparation and

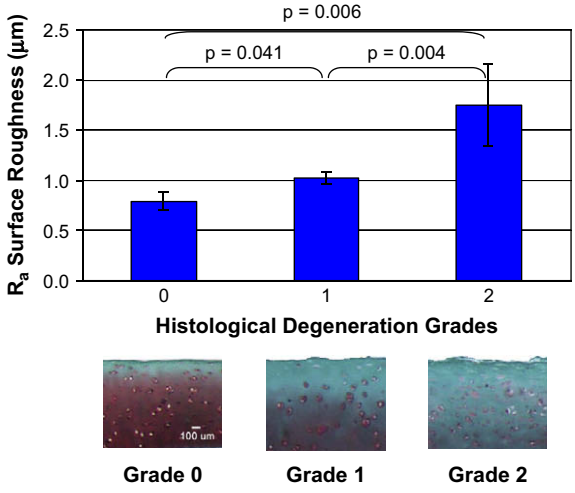

Fig. 5. Surface roughness of osteoarthritic cartilage relates to the histological grading. Top: The roughness values increase with increasing histological grades. Data are expressed as the mean ± standard error. Bottom: Representative histological sections for the three grades.

interferometric measurements can be performed under sterile conditions, as needed for tissue cultures. Fast, nondestructive, and benign to cartilage viability, the interferometric technique is thus suitable for monitoring topographical changes during experiments of *ex vivo* cartilage.

The interferometric technique has a low intrinsic coefficient of variance ("variability"), determined to be $<2\%$ of the R_a surface roughness. Thus, a 4% difference in R_a between two spots could be detected at the 95% confidence level with a power of 0.8 with only four interferometric measurements at each spot, given that only the microscope contributes to the standard deviation. More often, the surface roughness values of two or more areas are compared, in which case the intra-area variability comes into play. Here the intra-explant variability was approximately 17%. Because the total variance for a measurement is the sum of the variances from the various uncorrelated sources of variability, the contribution of the intrinsic term to the total variance is less than $2^2/17^2$ or 1.4%, corresponding to a contribution of less than 1% to the overall standard deviation of the measurement. It may therefore be concluded that under most situations the interferometry technique will contribute negligibly to the variance of the surface roughness measurements.

We are not aware of any reports on the intrinsic variability of stylus profilometry on cartilage. Because stylus profilometry leaves tracks, repeated measurements in the same location may significantly impact the surface roughness values, making it difficult to obtain a meaningful measure of intrinsic variability for this technique.

A limitation of the interferometric technique is that, in contrast to the profilometric technique, it requires proper surface preparation to remove discrete fluid films that may introduce optical artifacts. Another limitation is the fairly small size of the field of view, confined to $0.7 \times 0.5\ mm^2$ in our study due to the inherent waviness and irregularity of articular cartilage combined with the limited vertical scan (150 μm) of the microscope. This limitation can be overcome by performing multiple measurements in the region of interest. Stitching of adjacent fields to obtain a larger contiguous area is possible but may be restricted by the substantial curvature typical of many articular cartilage surfaces. Excessive curvature makes it necessary to repeatedly focus the microscope as it travels across the area. It may also require an even smaller field of view than used here. For many applications, however, these limitations will be outweighed by the speed and the noncontact, nondestructive nature of the technique.

Applying the capability of the interferometric technique to measure the topography of live cartilage, we made the interesting finding that cartilage surface roughness on the bovine distal femur is location dependent. Thus, the roughness values of explants from the medial aspect and from the trochlear groove were higher than those of explants from the lateral aspect and the condyles, respectively. Even though the structural properties and load-bearing regions of articular cartilage have been reported to vary with location within the joint[12,31], this is to our knowledge the first report of a quantified location-dependent variation in cartilage roughness. The implications of these results need to be further investigated. The roughness variations may be connected with the loads and motions to which the cartilage is subjected. Although location has not been a consideration in some recent lubrication studies of bovine knee cartilage[32,33], our findings suggest it may be a significant factor.

Histological examination of osteoarthritic cartilage can reveal surface characteristics such as fibrillation, discontinuity, vertical fissures and delamination[34]. As these characteristics increase the roughness of a surface, osteoarthritic cartilage is expected to have a higher roughness than healthy cartilage. During the investigation of lubrication mechanisms of the synovial joints, it was noted that osteoarthritic cartilage had as much as five times higher surface roughness of healthy cartilage[7]. Visual and histological examinations of osteoarthritic tissue have also confirmed the observation that disease changes the surface topography of articular cartilage[30,35,36]. Our findings of increasing surface roughness with histological degeneration score in human tali with cartilage degeneration characteristic of that seen in osteoarthritis are consistent with these observations. Moreover, the interferometric technique offers the possibility of systematically quantifying relationships of surface topography with histological observations of surface morphology and subsurface structure.

The interferometric technique developed here is expected to be applicable across species, given the inter-species similarity of cartilage in terms of composition, thickness, metabolic activity and functional response[18]. Indeed, in the present study the technique was successfully applied to perform topographical measurements of young, healthy bovine cartilage, as well as of mature, diseased human cartilage.

Conflict of interest

Vivek K. Shekhawat, Michel P. Laurent, Carol Muehleman and Markus A. Wimmer do not have a conflict of interest in connection with this work.

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