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Qualitative and quantitative assessment of cartilage degeneration using full-field optical coherence tomography *ex vivo*

R. Pailhé † ‡ §, A. Mounier § ‖ ¶, B. Boisson #, R.C. Rouchy § ‖ ¶, S. Voros † ††, E. Chipon § ¶ ‡‡, I. Boudry § ¶ ‡‡, M. Medici § ¶ ‡‡, C. Hughes † § ‡‡, A. Moreau-Gaudry † § ‡‡ §§ *

† *Université Grenoble-Alpes, CNRS, Grenoble INP, TIMC-IMAG, F-38000 Grenoble, France*
‡ *CHU Grenoble-Alpes, South Teaching Hospital, Department of Orthopaedic Surgery and Sport Traumatology, F-38130 Echirolles, France*
§ *INSERM, CIC 1406, F-38000 Grenoble, France*
‖ *CHU Grenoble-Alpes, Clinique Universitaire de Radiologie et Imagerie Médicale, F-38000 Grenoble, France*
¶ *Université Grenoble-Alpes, CIC 1406, F-38000 Grenoble, France*
# *CHU Grenoble-Alpes, Département d'Anatomie et de Cytologie Pathologiques, F-38000 Grenoble, France*
†† *INSERM, TIMC-IMAG, F-38000 Grenoble, France*
‡‡ *CHU Grenoble-Alpes, Pôle Recherche, F-38000 Grenoble, France*
§§ *CHU Grenoble-Alpes, Pôle Santé Publique, F-38000 Grenoble, France*

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SUMMARY

Objective: The purpose of this study was to investigate the ability of full-field optical coherence tomography (FFOCT) to qualitatively and quantitatively evaluate cartilage degeneration using the qualitative evaluation of histology sections as the reference.

Design: Thirty-three human knee cartilage samples of variable degeneration were included in the study. A closely matching histology and FFOCT image was acquired for each sample. The cartilage degeneration was qualitatively evaluated by assigning a grade to each histology and FFOCT image. The relevance of the performed grading was assessed by calculating the intra- and inter-observer reproducibility and calculating the concordance between the histology and FFOCT grades. A near-automatic algorithm was developed to quantitatively characterize the cartilage surface in each image. The correlation between the quantitative results and the reference qualitative histology was calculated.

Results: An almost perfect agreement was achieved for both the intra- and inter-reproducibility of the histology and FFOCT qualitative grading ($\kappa \geq 0.91$). A high and statistically significant level of agreement was measured between the histology and FFOCT grades ($W = 0.95$, $P < 0.05$). Strong and statistically significant correlations were measured between the quantitative results and the reference qualitative histology grades ($\rho \geq 0.75$, $P < 0.05$).

Conclusions: We have demonstrated that FFOCT is an alternative approach to conventional optical coherence tomography (OCT) that is as well adapted for the qualitative and quantitative assessment of human cartilage as the reference gold standard – histology. This study constitutes the first promising results towards developing a new diagnostic tool in the field of osteoarthritis.

Introduction

For *in vivo* cartilage assessment, arthroscopy is considered to be the clinical reference standard[1]. However, the grading depends on the surgeon's subjective interpretation, resulting in poor inter-observer reliability[2,3].

Optical coherence tomography (OCT) has been proposed as a means to overcome the limitations of conventional arthroscopy[1,4,5]. The relevance of OCT for human knee cartilage diagnosis has been evaluated with promising results[6–12] and a high level of agreement

* Address correspondence and reprint requests to: Pr. A. Moreau-Gaudry, Centre Hospitalier Universitaire Grenoble - CIC1406, Pavillon Taillefer, CS 10217, 38048 Grenoble - cedex 09, France

E-mail addresses: rpailhe@chu-grenoble.fr (R. Pailhé), amounier1@chu-grenoble.fr (A. Mounier), bboisson@chu-grenoble.fr (B. Boisson), rcrouchy1@chu-grenoble.fr (R.C. Rouchy), sandrine.voros@univ-grenoble-alpes.fr (S. Voros), echipon@chu-grenoble.fr (E. Chipon), iboudry@chu-grenoble.fr (I. Boudry), mmedici@chu-grenoble.fr (M. Medici), chughes@chu-grenoble.fr (C. Hughes), alexandre.moreau-gaudry@univ-grenoble-alpes.fr (A. Moreau-Gaudry).

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with histology has been demonstrated[7]. Due to the observer-dependent nature of qualitative grading, recent research activity has focused on quantitative OCT-based image analysis in order to achieve a more objective and reliable assessment of cartilage degeneration[1,8,9,11,13].

It has been demonstrated that the micrometre scale is most suitable for osteoarthritis (OA) assessment even though human knee cartilage surface alterations start at the nanometre scale[14]. Nevertheless, as OCT has an axial resolution of the order of 10–20 μm, which is comparable to low power histology, such an imaging tool is not able to provide the same level of tissue assessment as histology[9] and is therefore not optimal for OA assessment.

Improved cartilage assessment may be possible using full-field optical coherence tomography (FFOCT) which is an alternative, conceptually different, approach to traditional OCT. FFOCT is based on the principle of white light interference microscopy, with an optical arrangement based on a Linnik-type interferometer that enables 'en face' images to be acquired[15]. The FFOCT axial resolution (~1 μm) is of the order of 10 times higher than that achieved by traditional OCT systems (10–20 μm)[15], and is much closer to the resolution used for histology analysis. Promising results with this alternative approach to conventional OCT have already been published in a wide range of medical fields, including dermatology, gynaecology and oncology[16–19]. Nevertheless, to the best of our knowledge, this is the first time that FFOCT has been used for cartilage assessment.

The objective of this study was to investigate the ability of FFOCT to qualitatively and quantitatively evaluate human cartilage *ex vivo*. Closely matching histology and FFOCT images of human knee cartilage samples of variable degeneration were acquired. The ability of FFOCT to provide high quality images that enable the classification of cartilage degeneration was then evaluated (1) qualitatively using an established histology scoring system and (2) quantitatively using a near-automatic algorithm based on ISO standards.

Materials and methods

Study design and sample collection

This study was designed as a pilot, non-interventional, monocentric, prospective study, in accordance with the ethical standards of the responsible committee on human experimentation (Comité de Protection des Personnes, Sud-Est V, France, 16-RNI-07 and 16-RNI-08, 07/12/2016) and with the Helsinki Declaration of 1975, as revised in 2000. Thirty-three cartilage samples were harvested from the distal femoral cut of the knees of 18 patients (11 right, 7 left, 1 knee per patient, maximum 1 sample per condyle) undergoing total knee arthroplasty procedures for medial femoro-tibial OA, after individual informed patient consent was obtained. The patients included 4 men and 14 women with further details in Table I. The samples measured 8 mm in diameter and approximately 5 mm in height and were stored in isotonic saline solution at 4°C for a maximum of 72 h.

Table I
Cartilage samples were harvested from the knees of 18 patients (4 men and 14 women) with age, height, weight and body mass index details as follows

	Median	[Q1; Q3]
Age (years)	68.5	[66.3; 72.0]
Height (cm)	160.0	[159.3; 170.0]
Weight (kg)	74.5	[68.0; 78.1]
Body mass index (kg/m^2)	27.9	[23.1; 29.9]

Histology preparation

The samples were fixed in 4% buffered formalin, decalcified using Luthra's solution (3.2% 11.7 M HCl and 10.0% formic acid) for 48 h, dehydrated using 70–100% ethanol and rinsed with xylene. The samples were cut in half along the diameter; one half of each sample was embedded in paraffin and the other half of the sample was discarded, see Fig. 1(a). From the paraffin-embedded cartilage samples, a 3 μm-thick histology section was cut and stained using Safranin O and Fast Green. The histology sections were digitised using a Leica Biosystems Aperio digital pathology slide scanner (Wetzlar, Germany) and saved in JPEG format with 0.25 μm × 0.25 μm pixel resolution.

FFOCT acquisition

FFOCT imaging was performed using a commercial FFOCT system (Light-CT Scanner, LLTech, France) that enables 'en face' (transverse) images of a sample to be generated with 0.78 μm axial resolution. The system uses a spatially and temporally incoherent low power light source (quartz-halogen Schott KL 1500 Compact, Mainz, Germany) and two 10×/0.3 numerical aperture water immersion objectives. The effective spectrum of the system is centred around 750 nm, with a full width at half maximum of 300 nm. The depth of penetration is of the order of 200 μm to 1 mm, depending on the tissue imaged. The native field of view measures 0.8 mm × 0.8 mm, but a motorized transverse stage allows multiple individual image fields to be stitched together, enabling a 27 mm diameter area to be imaged.

A FFOCT image of each paraffin-embedded cartilage sample was acquired after the histology section had been cut in order to ensure that the same plane and depth (within the range of 3 μm, the thickness of the histology section) of cartilage were represented in both the histology and FFOCT images. As paraffin creates significant artefacts in the FFOCT images, the paraffin was lightly melted to ensure the paraffin was removed from the surface of the sample. The sample was then positioned in the dedicated sample holder; a cover glass was placed on top and secured in order to gently flatten the sample. The sample holder was placed in the imaging platform and optical fluid was added to the cover glass surface to ensure that the objective was immersed. A FFOCT image of the surface of each sample was acquired, with the acquisition zone defined in order to include all of the cartilage sample. A rapid verification of the absence of artefacts in the acquired image confirmed the complete removal of paraffin from the surface of the sample. If artefacts were present, the paraffin was further melted and the image was reacquired. The acquisition time was approximately 7 min per sample. The FFOCT images were exported in DICOM format. Figure 1 shows the histology and FFOCT images acquired for two samples that represent (b, c) low and (d, e) high grade cartilage degeneration.

Qualitative analysis

The first objective of the qualitative analysis was to compare the matching histology and FFOCT images in order to qualitatively identify which structures visible in histology and necessary for grading can be identified in FFOCT images. The second objective was to qualitatively grade the histology and FFOCT images and then evaluate the grading system with respect to histology and FFOCT by calculating the intra- and inter-observer reproducibility. Finally, the qualitative FFOCT grading was evaluated with respect to the reference qualitative histology by calculating the concordance between the histology and FFOCT grades.

The cartilage degeneration was qualitatively evaluated using the Osteoarthritis Research Society International (OARSI) Osteoarthritis

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