Osteoarthritis and Cartilage 20 (2012) 988–996

Osteoarthritis and Cartilage

Clinical outcome of autologous chondrocyte implantation is correlated with infrared spectroscopic imaging-derived parameters

A. Hanifi †, J.B. Richardson ‡, J.H. Kuiper ‡, S. Roberts ‡[a], N. Pleshko †*[a]

† *Tissue Imaging and Spectroscopy Laboratory, Department of Bioengineering, Temple University, Philadelphia, PA, USA*
‡ *Robert Jones & Agnes Hunt Orthopaedic Hospital NHS Foundation Trust, (ISTM, Keele University and Arthritis Research UK Tissue Engineering Centre), Oswestry, Shropshire SY10 7AG, UK*

ARTICLE INFO

Article history:
Received 24 September 2011
Accepted 21 May 2012

Keywords:
Cartilage repair
Autologous chondrocyte implantation
Histology
Type II collagen
Proteoglycan
FTIR imaging spectroscopy

SUMMARY

Objective: To investigate whether Fourier transform infrared imaging spectroscopy (FT-IRIS), a modality based on molecular vibrations, is a viable alternative to histology and immunohistochemistry (IHC) for assessment of tissue quality and patient clinical outcome.
Methods: Osteochondral biopsies were obtained from patients (9–65 months post-surgery) who underwent an autologous chondrocyte implantation (ACI) procedure to repair a cartilage defect ($N = 14$). The repair tissue was evaluated histologically by OsScore grading, for the presence of types I and II collagen by IHC, and for proteoglycan (PG) distribution and collagen quality parameters by FT-IRIS. Patient clinical outcome was assessed by the Lysholm score.
Results: Improvement in Lysholm score occurred in 79% of patients. IHC staining showed the presence of types I and II collagen in all samples, with a greater amount of collagen type II in the deep zone. The amount and location of immunostaining for type II collagen correlated to the FT-IRIS-derived parameters of relative PG content and collagen helical integrity. In addition, the improvement in Lysholm score post-ACI correlated positively with the OsScore, type II collagen (IHC score) and FT-IRIS-determined parameters. Regression models for the relation between improvement in Lysholm score and either OsScore, IHC area score or the FT-IRIS parameters all reached significance ($p < 0.01$). However, the FT-IRIS model was not significantly improved with inclusion of the OsScore and IHC score parameters.
Conclusion: Demonstration of the correlation between FT-IRIS-derived molecular parameters of cartilage repair tissue and patient clinical outcome lays the groundwork for translation of this methodology to the clinical environment to aid in the management of cartilage disorders and their treatment.

Introduction

Damage or degeneration of cartilage is frequently associated with joint pain and with changes in the macromolecular structure and content of the primary cartilage components[1–5]. It is widely accepted that cartilage injuries do not heal spontaneously[1], which is related in part to the avascular nature of the tissue and low mitotic activity of chondrocytes[6]. A variety of approaches have been investigated to improve cartilage healing including microfracture[7], subchondral drilling[7], osteochondral grafting[8], bone-marrow stimulation and a one-step technique based on bone marrow-derived cell transplantation[9]. These techniques rely on the potential of non-differentiated cells located in the subchondral area to migrate into the defect region and to differentiate into active chondrocytes[10,11]. More cell based methods for the repair of articular cartilage have also been developed on the basis of autologous cartilage–bone transplants or transplantation of cultured autologous chondrocytes[8,12]. Autologous chondrocyte implantation (ACI) is being used increasingly to treat cartilage defects[13]. This procedure aims to stimulate autologous cells to synthesize the extracellular matrix components of articular cartilage and to generate a zonal structure similar to normal articular cartilage[14]. Brittberg *et al.*[12] applied the ACI technique clinically for the first time with good to excellent results for healing. A number of studies followed with similar results, suggesting that ACI is an effective procedure for healing cartilage defects of the knee[1,15–17].

Evaluation of the success of cartilage repair procedures can include assessment of tissue harvested from the repair site, and several scoring systems are in use for semi-quantitative histological tissue evaluations[18], including the ICRS II score[19], O'Driscoll score[20]

* Address correspondence and reprint requests to: N. Pleshko, College of Engineering, Temple University, Philadelphia, PA 19122, USA. Tel: 1-215-204-4280.
E-mail address: npleshko@temple.edu (N. Pleshko).
[a] Senior co-authorship shared by SR and NP.

1063-4584/$ – see front matter
http://dx.doi.org/10.1016/j.joca.2012.05.007

and OsScore[21]. In general, these scoring systems consider tissue integrity, proteoglycan (PG) staining, chondrocyte clustering, presence of hyaline cartilage, and integration of repair with surrounding tissue. In addition, the presence of type II collagen in repair tissue is an important indicator of successful articular cartilage repair[22,23], as mechanically inferior fibrocartilaginous tissue that contains type I collagen is often produced[24]. Therefore, immunohistochemical assessment of types I and II collagen is often performed as well[14,25]. The major drawback for both histological and immunohistochemical assessment is the requirement to harvest tissues for analysis, which limits these evaluations in a clinical scenario.

Fourier transform infrared imaging spectroscopy (FT-IRIS) has been used to characterize the structure, distribution and orientation of extracellular matrix molecules, including types I and II collagen and PG, in histological sections of connective tissues[26], and in particular, in diseased and regenerative or engineered cartilage[4,27–34]. Further, the use of infrared spectroscopic techniques *via* fiber optic enables assessment of tissues *in situ*, without biopsy[35,36]. Thus, the possibility of utilizing these methods to monitor cartilage repair tissue quality, and thereby assist in disease management, is attractive. In particular, if parameters derived from infrared spectral characterization of repair tissue correlate with traditional histology-derived scores or with patient clinical outcome, FT-IRIS would provide a means to non-destructively assess the tissue status and could perhaps predict success of the repair procedure.

To date, there has been limited success in correlating the clinical outcome of patients who have undergone cartilage repair procedures with the quality of the repair tissue formed[21,23]. The aims of the current study were, therefore three-fold, namely (1) to investigate the molecular properties of cartilage repair tissue post-ACI procedure using FT-IRIS, (2) to assess correlations between FT-IRIS-derived properties and the gold-standard techniques of histological grading and type II collagen IHC and (3) to investigate if FT-IRIS is a viable alternative to histological or immunochemical assessment by exploring the relationship between histological, immunohistochemical, or FT-IRIS assessment of tissue quality and clinical outcome.

Materials and methods

Tissue

Patients, aged 28–53 years ($N = 14$), with chondral or osteochondral defects on the femoral condyles or trochlea, were treated with ACI according to the technique of Brittberg *et al.*[12]. Full-depth core biopsy samples (1.8 mm in diameter) were obtained from patients who had undergone ACI 9–65 months previously. In addition, tissue from a healthy cadaveric knee (40-year-old) with macroscopically normal cartilage was obtained within 24 h of death from the UK Human Tissue Bank. A biopsy was obtained from the medial femoral condyle, similar to the region most commonly treated with ACI. All tissues obtained were under approval of the local Research Ethical Committee. The tissues were immediately snap frozen and stored in liquid nitrogen until processed.

Clinical outcome (Lysholm score)

Patient clinical outcome was evaluated based on the Lysholm score[37,38]. The Lysholm scale is a well-validated functional and pain score designed for knee injuries that has been utilized to assess the clinical outcome of ACI procedures[37,39] and ranges from 0 (poor outcome) to 100 (best). The parameters that comprise the Lysholm score are summarized in Table I[40]. For each patient, the Lysholm score was assessed at the time of surgery (pre-operatively) and at the time of biopsy; the difference between these two values was reported as the actual improvement in Lysholm score, or percent Lysholm improvement.

Table I
Parameters evaluated in the Lysholm score (total 100 points)[40]

Parameter	Points
Limp	Five points
Support	Five points
Stair climbing	10 points
Squatting	Five points
Instability	25 points
Pain	25 points
Swelling	10 points
Locking	15 points

Histology and IHC analysis

Seven-micron thick cryosections of the harvested biopsies were stained with hematoxylin and eosin (H&E) for histologic evaluation. The semi-quantitative OsScore was utilized for assessment of tissue quality[21], and ranges from 0 (worst) to 10 (best), based on the following features of the repair tissue: cartilage type present (hyaline cartilage, fibrocartilage or fibrous tissue), integrity of the tissue surface, degree of metachromasia (toluidine blue or safranin O staining), formation of chondrocyte clusters, presence of blood vessels or mineralization, and integration with the calcified cartilage and underlying bone.

Immunohistochemical staining was performed on cryosections using antibodies specific to types I or II collagen. Separate tissue sections were incubated for 1 h at room temperature with primary antibodies against type I collagen (monoclonal antihuman, clone no 1-8H5; ICN) and against type II collagen (CIICI; Developmental Studies Hybridoma Bank, Iowa, USA). The type II collagen stained IHC images were scored in two ways. A semi-quantitative assessment of the percentage of area of the section of cartilage tissue which was immunostained for type II collagen, termed 'IHC Area score', was obtained using image analysis software (NIS-Elements Basic Research, Nikon, Japan)[14]. A second more detailed assessment of the staining intensity in defined regions was also undertaken on a number of samples and used to determine the relationship of the IHC intensity (termed as 'IHC Intensity score') with FT-IRIS-derived parameters. For this, each tissue section was divided into nine regions and each region graded from 0 to 3 based on staining intensity, where grade 0 represents the minimum amount of type II collagen staining (white) and grade 3 represents the maximum amount of type II collagen immunostaining (dark brown).

FT-IRIS data acquisition

FT-IRIS data were acquired in the mid-infrared region, 4000–800 cm^{-1}, at 8 cm^{-1} spectral resolution and 25 μm spatial resolution, with two co-added scans per pixel, using a Spectrum Spotlight 400 FT-IR imaging spectrometer (Perkin Elmer, CT, USA). Polarized FT-IRIS data were collected with an infrared polarizer inserted in the beam path. Data collection time was approximately 30 min/sample.

FT-IRIS data processing

FT-IRIS images were created based on vibrational absorbances for the specific molecular components of interest in cartilage using ISys software v5.0 (Malvern Instrument, Columbia, MD, USA)[28].