Medical Engineering & Physics 36 (2014) 850–858

Contents lists available at ScienceDirect

Medical Engineering & Physics

journal homepage: www.elsevier.com/locate/medengphy

Poroelastic response of articular cartilage by nanoindentation creep tests at different characteristic lengths

M. Taffetani [a], R. Gottardi [c], D. Gastaldi [a], R. Raiteri [b], P. Vena [a,d,*]

[a] Department of Chemistry, Materials and Chemical Engineering, Laboratory of Biological Structure Mechanics (LaBS), Politecnico di Milano, Italy
[b] Department of Informatics, Bioengineering, Robotics and System Engineering, University of Genova, Genova, Italy
[c] Ri.MED Foundation, Palermo, Italy
[d] IRCCS, Istituto Ortopedico Galeazzi, Milano, Italy

ARTICLE INFO

Article history:
Received 20 April 2013
Received in revised form 14 February 2014
Accepted 23 March 2014

Keywords:
Nanoindentation
Articular cartilage
Time-dependence
Poroelasticity, Size effect

ABSTRACT

Nanoindentation is an experimental technique which is attracting increasing interests for the mechanical characterization of articular cartilage. In particular, time dependent mechanical responses due to fluid flow through the porous matrix can be quantitatively investigated by nanoindentation experiments at different penetration depths and/or by using different probe sizes. The aim of this paper is to provide a framework for the quantitative interpretation of the poroelastic response of articular cartilage subjected to creep nanoindentation tests. To this purpose, multiload creep tests using spherical indenters have been carried out on saturated samples of mature bovine articular cartilage achieving two main quantitative results. First, the dependence of indentation modulus in the drained state (at equilibrium) on the tip radius: a value of 500 kPa has been found using the large tip (400 μm radius) and of 1.7 MPa using the smaller one (25 μm). Secon, the permeability at microscopic scale was estimated at values ranging from 4.5×10^{-16} m^4/N s to 0.1×10^{-16} m^4/N s, from low to high equivalent deformation. Consistently with a poroelastic behavior, the size-dependent response of the indenter displacement disappears when characteristic size and permeability are accounted for. For comparison purposes, the same protocol was applied to intrinsically viscoelastic homogeneous samples of polydimethylsiloxane (PDMS): both indentation modulus and time response have been found size-independent.

1. Introduction

Articular Cartilage (AC) is a connective tissue able to grant a low friction coefficient [1] and the smoothness in the transmission of load between diarthrodial joints. AC inhomogeneous, highly anisotropic and non-linear properties are strictly related to its structure, which is in turn determined by the molecular and ultrastructural organization of its components [2].

The solid phase of AC is constituted mainly by a dense network of collagen fibrils and interspersed proteoglycans (PGs) with a sparse population of chondrocytes; the interstitial fluid phase, that saturates the solid matrix, is composed by water and free ions as Na^+, K^+, Ca^+ [3]. Since the superficial layer of AC is crucial for both its mechanical function and in damage initiation, there is a major interest in investigating the properties of the top 20% of the overall tissue thickness. The superficial layers present the higher water content, almost 80% of its wet weight, and collagen density, up to 50–75% of its dry weight. The viscoelastic properties, which are dependent on the molecular structure and its evolution under stress, and the extrinsic properties, which are dependent on the fluid flow through the porous solid matrix, exhibit mutual interaction.

In this work we are interested in the extrinsic time dependent properties of the AC tissue, governed by the fluid flow through its porous microstructure, i.e., in AC poroelasticity. Terzaghi [4] first introduced the poroelastic model for soils, assuming that both the solid and fluid phases fill homogeneously the volume, and Biot [5] extended it to a tridimensional anisotropic case; moreover, Rice and Cleary [6] highlighted the limit conditions described by drained (at equilibrium) and undrained (short term) moduli. A comprehensive description of the physics of fluid saturated solids is also available in Coussy [7]. The problem of compression of disks with anisotropic poroelasticity was solved analytically in Cowin and Doty [8]; the application of the poroelastic theory to cartilage can be found in

* Corresponding author at: Department of Chemistry, Materials and Chemical Engineering, Laboratory of Biological Structure Mechanics (LaBS), Politecnico di Milano, Italy. Tel.: +39 0223994236; fax: +39 0223994220.
E-mail address: pasquale.vena@polimi.it (P. Vena).

http://dx.doi.org/10.1016/j.medengphy.2014.03.008
1350-4533/

many papers including those by Armstrong et al. [9] and Cohen et al. [10]. The biphasic (or triphasic) models consider, instead, the different cartilage components separately [11].

At the macroscopic scale, a wide range of studies on AC behavior have been conducted using confined and unconfined compression tests and shear tests. Schinagl et al. [12] performed stress-relaxation confined compression tests on bovine cartilage indentifying heterogeneous elastic properties of the tissue. Newborn bovine patellofemoral groove AC was studied by Ficklin et al. [13] identifying strain dependent AC permeability. A wide spectrum of cartilage moduli from tension to compression was reported in the work of Chahine et al. [14] who performed unconfined compression and tensile tests. The compressive modulus was found nearly constant independently from the direction of load and increasing with the depth, whereas the tensile modulus was strongly affected by both load direction and depth.

The main disadvantages of the tests at macroscale is that the experimental setup can significantly affect the results through misalignments, non-ideal contact conditions between tissue and sample holder and boundary effects which can result in a under- or overestimation of the tissue elastic properties. Nanoindentation is a widely used experimental technique for the analysis of biological tissues in physiological conditions [15,16] with a relatively simple experimental set up. The technique is able to probe small amounts of material with respect to the sample size far from the sample boundaries; therefore, material properties of intact tissue can be obtained.

Nanoindentation can be applied using different sizes for the indenter, opening the possibility to investigate the structure from the whole tissue to the single components. In the work of Jin and Lewis [17], the estimation of the Poisson's ratio was performed using information extracted by two indentations with different tip sizes; in the work of Hu et al. [18], dimensionless relaxation function were introduced from the results of indentation tests carried out with different tips. Oyen et al. [21] have studied the size effect introduced through indentation tests on hydrated tissues as bone and cartilage. They proposed an effective fitting procedure to identify poroelastic and viscoelastic parameters from creep (and relaxation) master curves, and provided a physical interpretation of these parameters with special reference to bone. Park et al. [19] found an effective modulus increasing from 60 kPa to 160 kPa for up to 600 nm of probed depth using a sharp conical tip of radius and opening angle of 50 μm and 35°, respectively. The same depth is investigated with a spherical tip with radius of 2.5 μm, obtaining a modulus that increases from 20 kPa to 40 kPa. Galli et al. [20] used the nano- and microindentation techniques to characterize hydrogels in the time domain as well as in the frequency domain and discussed the time dependence induced by the extrinsic fluid-flow mechanisms in view of the length-scale effects related to the use of different probe sizes.

The aim of this paper is to provide a framework for the quantitative interpretation of the poroelastic response of AC subjected to a multiload spherical indentation test coupled with creep tests. The effect of the probed length and of the tissue strain on the poroelastic tissue response is investigated in detail. Assuming that the dissipation mechanism during creep nanoindentation experiment is primarily of extrinsic nature (i.e., poroelastic), the response at equilibrium, the short term response, and the permeability are investigated through experiments with different tip radii to highlight the dependence on strain. It will be shown that the experimental response is consistent with the above assumption. To this purpose, nanoindentation creep experiments have been also carried out on polydimethylsiloxane (PDMS) samples in dry environment, which are expected to have a time-dependent response of intrinsic nature (i.e., viscoelasticity).

2. Method

2.1. Nanoindentation testing

2.1.1. Samples preparation and instrumentation

AC samples are obtained from lateral and medial condyles of a knee of mature bovine. Three AC samples extracted from neighboring areas of the same bovine knee have been used for testing, one for each of the three test types. Samples harvesting is performed through a biopsy punch with inner diameter of 10 mm. Each explanted plug consisted of a full-thickness AC fragment with its underlying subchondral bone. The plugs are transferred in PBS (2.6 mM NaH_2PO_4, 3 mM Na_2HPO_4, 155 mM NaCl, 0.01% NaN_3 w/v, pH 7.0) supplemented with 20 μg/ml of gentamycin (Invitrogen, Carlsbad, CA, USA,) and a protease inhibitor cocktail (P8340, Sigma, St. Louis, MO, USA) and kept frozen at temperature of −80 °C until measurement; it is assumed the freezing does not affect the behavior of the tissue as described by Kiefer et al. [22].

PDMS disks are obtained mixing viscous components and curing agents with a ratio of 10:1 by weight using the commercial Sylgard 184 Elastomer Kit (Dow Corning, Midland, Michigan, USA). After mixing the two components for at least 5 min, cycles of applied vacuum and rest were carried out to remove all air bubbles. The mixture was then poured in a cylindrical mold of 15 cm of diameter and 0.5 cm of thickness and the PDMS samples were allowed to solidify at 65 °C for 45 min. A biopsy punch with inner diameter of 10 mm was then used to core smaller PDMS samples.

All experiments presented in this work are performed using a NanoTest Indenter (Micro-Materials Ltd., Wrexham, UK) equipped with a liquid cell able to keep samples in a hydrated and fully saturated state (Fig. 1). A 500 mN load cell with a force resolution of 30 nN has been used; the displacement sensor detects penetration depths up to 30 μm with a resolution of 0.1 nm.

Two spherical tips with different radii are used, $R_{400} = 400$ μm and $R_{25} = 25$ μm in the case of cartilage, and $R_{100} = 100$ μm and

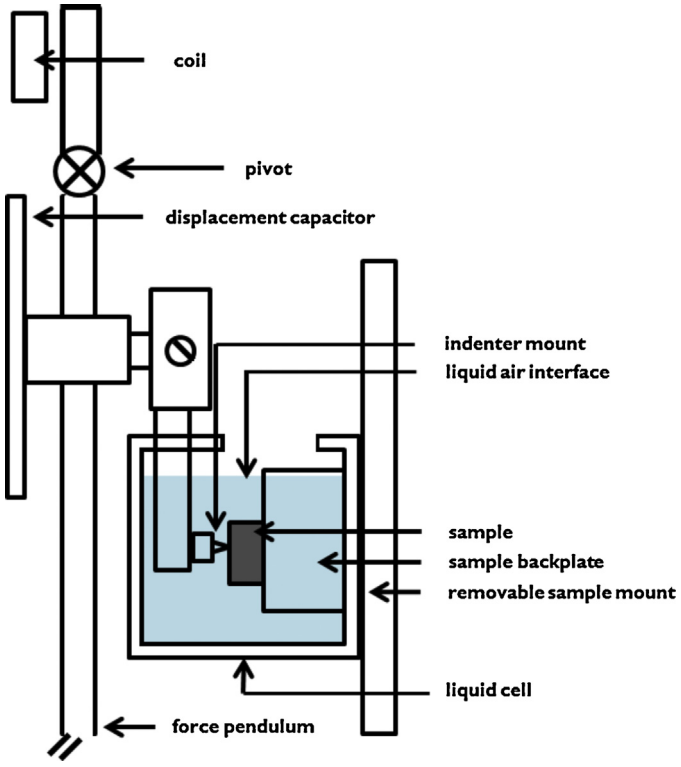

Fig. 1. Sketch of the experimental set-up adopted for the nanoindentation test.