Use of an optimized automatic procedure for measuring the hydraulic permeability of articular cartilage

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***Abstract*— Permeability, *k*, is one of the main characteristics that allows cartilage to perform its functions. We developed an automatic procedure, based on LabVIEW, to directly measure the permeability of cartilage by use of a custom-made permeation setup. We compared the results with those obtained by standard indirect methods based on the poroelasticity theory and with numerical predictions, obtained by a mathematical model developed under Comsol Multiphysics. The preliminary results demonstrate the effectiveness of our automatic method to measure the hydraulic permeability of articular cartilage or any other soft tissue.**

***Keywords*—Cartilage, osteoarthritis, permeability, poroelasticity**

I. Introduction

THE articular cartilage is a thin layer of specialized connective tissue with viscoelastic and mechanical properties, which are especially important for preserving joints as they reduce friction between the moving articular surfaces, they also allow for better load distribution and provide impulsive loads absorption.

Permeability, *k*, is one of the main characteristics that allows cartilage to perform its functions adequately and can be defined as the ease with which a fluid passes through a porous medium. In healthy articular cartilage, the low value of this property (10^{-16} $m^4/N{\cdot}s$) holds water inside the tissue, and this retained water is used to withstand most of the load to which the cartilage is subjected, thus preserving the ECM. This property also prevents synovial fluid from penetrating the cartilage tissue, ensuring adequate lubrication of the joints.

When the cartilage tissue undergoes degenerative processes like osteoarthritis (OA), an initial chondrocyte proliferation occurs, followed by an increase of water content and loss of proteoglycans and glycoproteins (GAG), which ultimately lead to breakage of the collagen fibers and result in superficial pitting. The gradual decrease of proteoglycans, responsible for the water content of the matrix, entails an increase of permeability with consequent loss of the normal functions of the healthy tissue.

II. Materials and Methods

In this study we analysed 14 OA cartilage samples derived from 2 human femoral heads that had been removed during hip replacement surgery. The samples underwent direct and indirect measures (stress-relaxation test), as permeability can be directly measured, exploiting the Darcy equation, or it can be indirectly estimated, using theoretical models.

A. Set-up and optimized automatic procedure

Fig.1A represents our automated experimental set-up, used to facilitate the direct permeability measurement, composed by a custom made chamber (where the sample is housed, Fig. 1B), a volumetric pump (NE500, New Era Pump Systems, Inc., USA), to control the flow rate, a pressure transducer (IMP, Sensors One Ltd, UK), a digital camera (Dino-Lite, Taiwan) to measure the effective flow through the sample, and the control software developed within LabVIEW (National Instruments), to make the test procedure automatic.

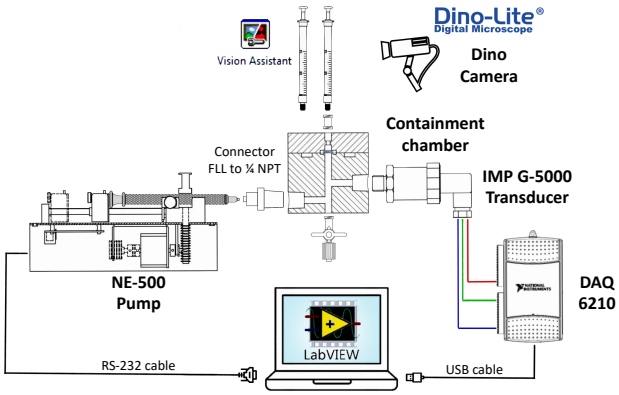

Fig. 1A: General scheme of the set-up that represents all of the components and their connections: the fundamental part is the control software which can acquire, through the DAQ acquisition card connected to the computer, the pressure signals measured by the transducer and control the syringe pump connected to the sample containment device; to estimate the effective flow rate through the sample, a video camera and two graduated capillaries are connected to the permeation chamber.

LabVIEW is a graphic programming environment that enables the design of clear, simple and intuitive interfaces and allows the direct control of the electronic instrumentation, such as the data acquisition hardware (DAQ), for the analysis and elaboration of signals.

Our program is able i) to control the volumetric pump, ii) to acquire, through the DAQ system, the pressure signals measured by the transducer and iii) to increase the flow rate value (input variable), when the signal pressure remains in a range previously defined by the user (Pressure stab. range±) during a specific amount of time (Stabilization time). The program can also record and automatically save the set parameters and the pressure values in an external Excel file at the end of the test, this feature allows the user to analyse and elaborate the output data at a later time to calculate the permeability sample.

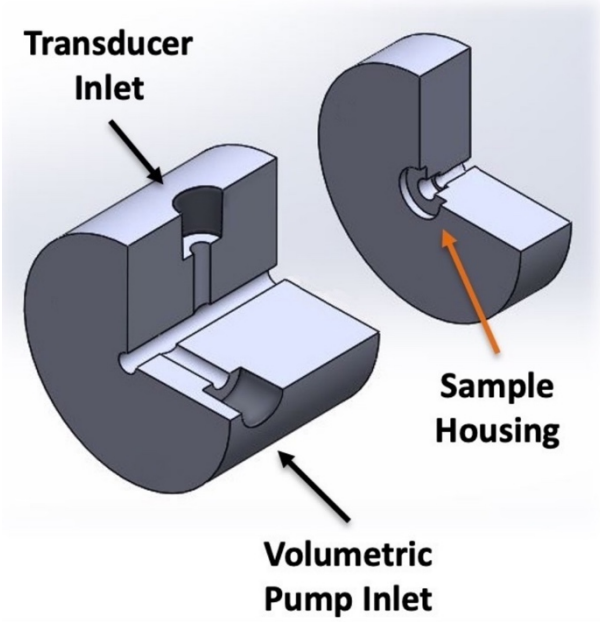

Fig. 1B: A detailed view of the custom permeation chamber composed by two axial cylinders. The sample housing is 12 mm in diameter whereas the area available for flow is 5 mm in diameter.

More useful functions performed by the implemented software are *a. Calibration of the pressure transducer* implemented directly into the control interface; *b. Storage of the runtime pressure values*, useful to obtain the pressure trend during the entire test and facilitate the data post-processing. Data are saved in real-time, based on a time-sampling chosen by the user; *c. Step independent flow rate increments*, this feature makes the test settings more versatile; *d. Maximum time step*, the purpose of this function is to limit the duration of the entire test, in fact, the excessive test length may cause pressure instability; *e. Pressure conversion* used to visualize the pressure value in the desired units of measurement; *f. Target Pressure*, whose purpose is to protect the pressure transducer and the analysed sample from damage: if the pressure value reaches the *Target Pressure* the test is instantly interrupted.

B. Direct permeability measurement

We performed 3-5 measurements for each sample, setting the flow rate (value range from 15 to 200 µL/hr) and measuring the pressure value when stabilized.

Permeability was computed as a function of the pressure gradient using Darcy equation from the parameters and the pressure values saved at the end of the test:

$$k = (A \cdot \Delta p) \,/\, (s \cdot Q) \qquad (1)$$

where A= passage area [m^2]; s= thickness of sample [m].

C. Indirect permeability measurement

The central 5mm diameter of the samples tested for permeation were cut and subjected to indirect permeability measurements, via confined compression test in movement control (stress-relaxation test), with 5 deformation ramps. We interpolated the resulting experimental curve with the analytical solution extracted from Mow's linear biphasic model [1], thus obtaining the aggregate module H_A and the axial permeability value. The mean H_A value of each sample was exploited to extract the deformation levels of the direct tests through the relation [2]:

$$\varepsilon = \Delta p / 2H_A \qquad (2)$$

this step allowed us to find the k(ε) trend of both tests.

The experimental data were interpolated with a decreasing exponential according to Lai & Mow law [3]:

$$k = k_0 e^{M_0 \varepsilon} \qquad (3)$$

D. GAG evaluation

After the permeability measurements, for each sample the GAG and DNA contents were evaluate as described in [4]. Briefly, specimens were minced and digested in proteinase K (pH 7.6) (Sigma-Aldrich, Milan, Italy), then centrifuged and the supernatant was collected for analyses. DNA content was measured in the supernatant using a cell proliferation assay (CyQuant® kit – Life Technologies, Monza, Italy). The quantification of sGAG was performed using the 1,9-dimethylmethylene blue (DMMB) dye-binding assay (Sigma-Aldrich, Milan, Italy) and compared to a standard curve of chondroitin sulphate (Blyscan – Biocolor, Magenta, Italy). Briefly, the samples were incubated in 40 mM glycine/NaCl (pH 3.0) with 16 mg/mL DMMB at RT. The DNA and sGAG concentrations were determined by means of a spectrophotometer (Perkin Elmer Victor X3 microplate reader). Data from DNA and sGAG content analysis were normalised to the sample dry weights.

The mean value and standard deviation of H_A, k_0 for direct (k_{0darcy}) and indirect measurements (k_{0cc}) were calculated for each patient, to investigate the presence of a possible correlation between these parameters and the GAG content.

E. COMSOL Multiphysics simulations

Numerical simulations were performed under COMSOL Multiphysics 5.4 (COMSOL Inc., Burlington, MA, USA) to reproduce the conditions of the direct permeability test conducted on the cartilage tissue and design better experiments. In particular, the purpose of these simulations was to quantify the effect of several parameters on the stabilization time of the upstream pressure.

Time dependent studies were performed by means of the Poroelasticity module, as it combines a transient formulation of Darcy's law with a quasi-static formulation of Solid Mechanics, based on the resolution of Navier-Stokes equations, which together constitute a multiphysics coupling suitable to represent soft tissues.

Having to represent a cylindrical specimen, a two-dimensional axisymmetric model was created, which translates into a rectangular geometry, having dimensions equal to the thickness and radius of the sample (Fig. 2). Given the regularity of the implemented geometry, a mapped mesh consisting of square elements was created; moreover, since the variable of interest is more significant on the upper boundary, it was decided to build a finer mesh in the upper part of the geometry by creating a regular distribution of the elements in length with a ratio of 0.1 in thickness, as visible in Fig. 2.

Specifically, the steady state pressure of the upper side of the rectangle is the representative variable of the problem and the simulations were conducted varying the parameters of initial velocity v_0, permeability k, elastic module E and sample

thickness *s*. The values of the parameters were chosen on the basis of the experimental results obtained and are shown in Table 1.

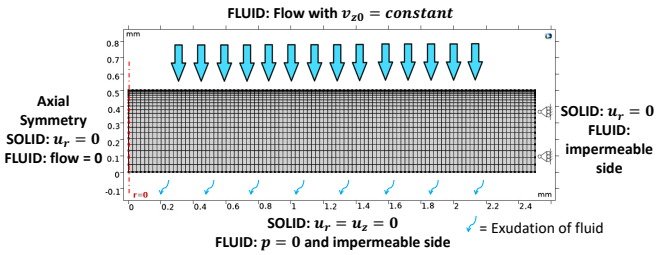

Fig. 2: Mesh and boundary conditions used in simulations.

TABLE I
SIMULATION PARAMETERS

Parameter	*Values*		
s [mm]	0.5	1	1.5
E [MPa]	0.1	0.3	0.6
v_0 *[m/s]*	2.83e-8	1.47e-7	2.83e-7
k [m^2]	1e-17	1e-18	1e-19

Values used to perform parametric simulations.

III. RESULTS

A. Permeability Measurements

Pressure drops measured across the samples varied from 0.2 to 1 atm, depending on the sample physical and mechanical properties (k, s, E).

For each sample, the comparison graphs of direct and indirect permeability as a function of deformation were performed. Fig.3 shows one example of permeability trends where all points, i.e. those computed from the model and those derived from the experiments, were fitted by an exponential curve. For both tests, the permeability decreases with increasing deformation.

The average and SD values for indirect and direct k_0 and for H_A are conformed to the ones found in the literature [5]–[8] and they are summarized in Table 2.

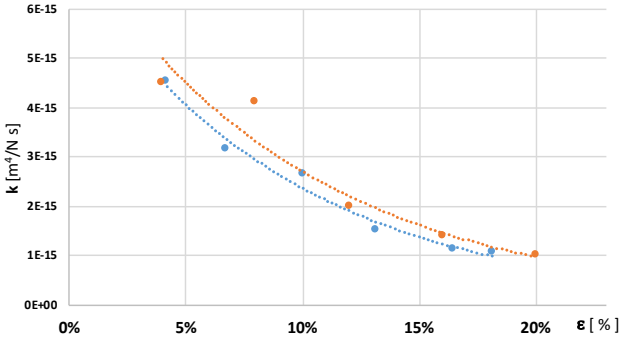

Fig. 3: Comparison between the direct and indirect test k(ε) curves for one of the tested cartilage samples. Blue dots and line: direct measurements (Darcy law); orange dots and line: indirect measurements (confined compression).

TABLE II
AVERAGE AND SD VALUES

Tissue	k_{0darcy} *[m^4/(N·s)]*	k_{0cc} *[m^4/(N·s)]*	H_A *[MPa]*
OA cartilage	0.36±1.8e-14	0.96±1.29e-13	0.36±0.25

Permeability values obtained by direct (k_{0darcy}) and indirect (k_{0cc}) test and aggregate modulus for analyzed cartilage.

In a few cases the two intrinsic permeability values differed by even by an order of magnitude, in general the indirect values where higher than the direct values. Causes of such differences can be attributed to intrinsic confined compression test errors [6], as the ideal boundary conditions do not coincide to the ones that occur during the effective test (gap between piston and chamber, imperfect sample dimensions). Moreover, the lengthy duration of the direct measurement test deteriorates the analysed sample, making it swell and then resulting in permeability increase measured indirectly.

B. GAG evaluation

As seen in literature, we found a mild positive correlation between GAG and H_A [7], [10] and a weak negative correlation between GAG and k_{0darcy} [10], [11]. Contrary to expectations, the relation between GAG and k_{0cc} resulted in a weakly positive correlation [7]. This phenomenon can be explained considering the sample swelling and the strong positive correlation between water content and permeability [12]–[14].

C. COMSOL Multiphysics simulations

The results of parametric simulations, summarized in Fig. 4, show that the stabilization time during permeation tests is strongly affected by the tissue parameters, while the test settings are irrelevant. In particular, the regime time is independent from the inflow velocity v_0, and it is instead non-linearly influenced by the permeability *k*, the elastic modulus *E*, and the sample thickness *s*. As shown in Fig. 4, the regime time decreases with the increase in permeability and elastic modulus, while increases with the increase of the sample thickness. These numerical results confirm how samples of soft tissues with low intrinsic permeability and stiffness typically show very high stabilization times.

Considering our proposed set of parameters, the permeability is the parameter that most influences the stabilization time: the latter decreases of two orders of magnitude when *k* increases from 1e-19 to 1e-17 m^2.

On the basis of these simulations, we believe it is inappropriate to maintain the same settings for different samples in the direct tests of permeability.

IV. CONCLUSION

The preliminary results demonstrate the effectiveness of our automatic method to directly measure OA cartilage permeability [7], [8]. A mild positive correlation between GAG e H_A and a weak negative correlation between GAG and k_0 for direct measurements [10], [11] was found in agreement with other authors [8], [10], [11], whereas GAG and k_0 for indirect measurements resulted in a weakly positive

correlation, probably because of sample swelling during permeation tests [12]–[14].

Finally, we hypothesised a new test protocol, which entails an inverted order of the test execution (contrary to what has been done in this preliminary study): if the confined compression test is carried out first, the direct permeability measure can be more robust without affecting the direct measurement test.

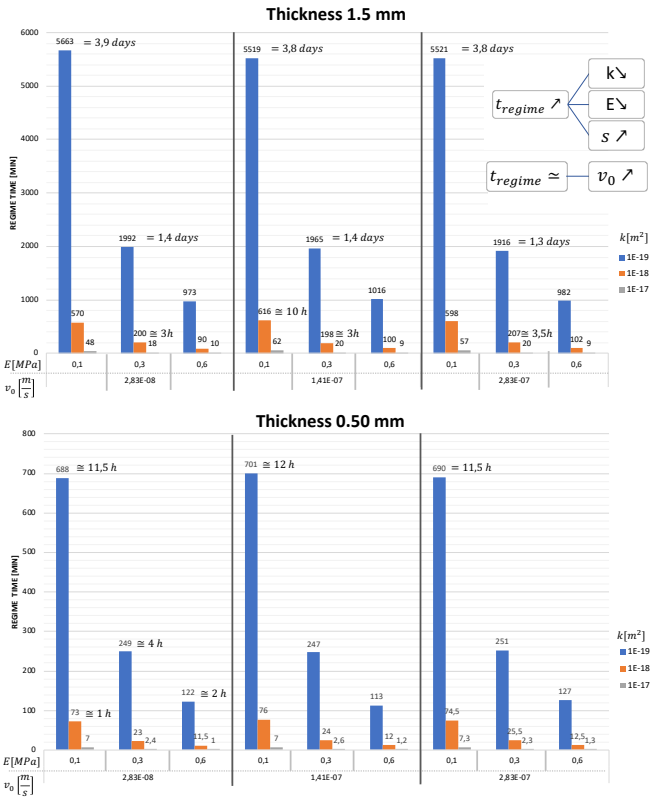

Fig. 4: Simulation results for 1.5 mm and 0.5 mm sample thickness. The time requested to reach a constant upstream pressure is calculated for different values of flow velocity (v_0), permeability (k), elastic modulus (E) and sample thickness (s).

This new executive order could give us two benefits: on one side, the measurement errors caused by the sample swelling and deterioration that occur during the direct permeability measurement test would be eliminated. On the other hand, the H_A value estimated from the indirect permeability measurement would allow us to determine the maximum pressure value that can be reached during the direct permeability tests, in order to guarantee the hypothesis of linearity required to apply Eq. 2.

This maximum pressure value can be entered into the direct test control software, under the voice Target Pressure, to ensure that the obtained result (i.e. k(Δp) and then k(ε)) satisfies the analytical model.

The setting of an efficient maximum time length of a single step (t max step) is extremely important: to do this, a numerical simulation should be run, using the parameters of the sample analyzed via a confined compression test to set the simulation, before the conduction of a direct measurement test.

Specifically, the model simulated using Comsol Multiphysics should have the same thickness of the analyzed sample, its elastic module E should be derived from Poisson ratio and the aggregate module H_A, while its permeability value (deducted from the indirect measure) may be decreased by 30% to take into account the confined compression test intrinsic errors [6].

In a future perspective, after the validation of the new execution order, this new test protocol could be used in combination with the optimized set-up to fulfil a wider range of applications, for example to compare the properties between healthy and pathological tissue or to complete the permeability study, analysing its trend also as a function of a constant pre-clamping deformation.

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