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Short communication

Experimental method to characterize the strain dependent permeability of tissue engineering scaffolds

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ABSTRACT

Permeability is an overarching mechanical parameter encompassing the effects of porosity, pore size, and interconnectivity of porous structures. This parameter directly influences transport of soluble particles and indirectly regulates fluid pressure and velocity in tissue engineering scaffolds. The permeability also contributes to the viscoelastic behavior of visco-porous material under loading through frictional drag mechanism. We propose a straightforward experimental method for permeability characterization of tissue engineering scaffolds. In the developed set-up a step-wise spacer was designed to facilitate measurement of the permeability under different compressive strains while maintaining similar experimental conditions during the successive measurements. As illustration of the method, we measured the permeability of scaffolds presenting different average pore sizes and subjected to different compression values. Results showed an exponential relationship between the permeability and the average pore size of the scaffolds. Furthermore, the trend of the permeability decrease with compressive strains was depending on pore sizes of the scaffolds. The permeability also appeared to play a role in relaxation behavior of the scaffolds.

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1. Introduction

Permeability is considered as an integrative variable for tissue engineering (TE) scaffolds reflecting the role of contributing parameters in their architectures such as porosity, pore size, interconnectivity and orientation of pores to flow direction (Li et al., 2003). The permeability of the scaffold not only influences infiltration and diffusion of soluble particles in TE applications but also passively controls the velocity and pressure fields of fluid content inside the scaffold under mechanical stimulation.

From another point of view, the permeability can be understood as a primary mechanical determinant defining frictional drag that is the resistance of a 3D biomaterial for flowing fluid through its tortuous structure (Mow et al., 1980; Nasrollahzadeh and Pioletti, 2016). Accordingly, permeability can be controlled to tune viscoelastic behavior of biomaterials under loading. Specifically, energy dissipation and stress relaxation behavior of TE scaffold are key viscoelastic features in dynamic and static loading regimes, respectively. Interestingly, these mechanobiological variables have been shown to be significantly influential on cellular response of load bearing tissues (Abdel-Sayed et al., 2014; Chaudhuri et al., 2016).

Obviously, permeability varied under compressive strain due to deformed scaffold internal structure and geometry. Different theoretical models have been developed to establish relationships between the strain and permeability for several tissues such as cartilage (interested reader is referred to (Lai and Mow, 1979; Lai et al., 1981; Holmes, 1985; Holmes and Mow, 1990)). While many experimental studies have been performed to measure the permeability of biological tissues and scaffolds (see (Pennella et al., 2013) for a review), only very few techniques have been developed to quantify the corresponding strain dependent permeability of the scaffolds. In a pioneer work, O'Brien et al. (2007) developed an experimental set-up allowing to measure the permeability of highly porous natural polymeric scaffolds (porosity higher than 95%) under different strain conditions. However, their system necessitates disassembly and reassembly steps of the rig for measurements of the permeability under different compressive strains. This technical approach impacts the reliability of the permeability measurement as permeability quantification is highly dependent on the initial and boundary conditions.

Kenneth et al. (Ng et al., 2014) tested synthetic PVA scaffolds under confined compression to indirectly quantify strain dependent permeability according to the biphasic theoretical model for cartilage (Lai and Mow, 1979). The employed model for permeability determination in that study was based on linear infinitesimal strain theory, while the range of applied compressive strain caused large deformation and nonlinear behavior. Therefore, the predicted

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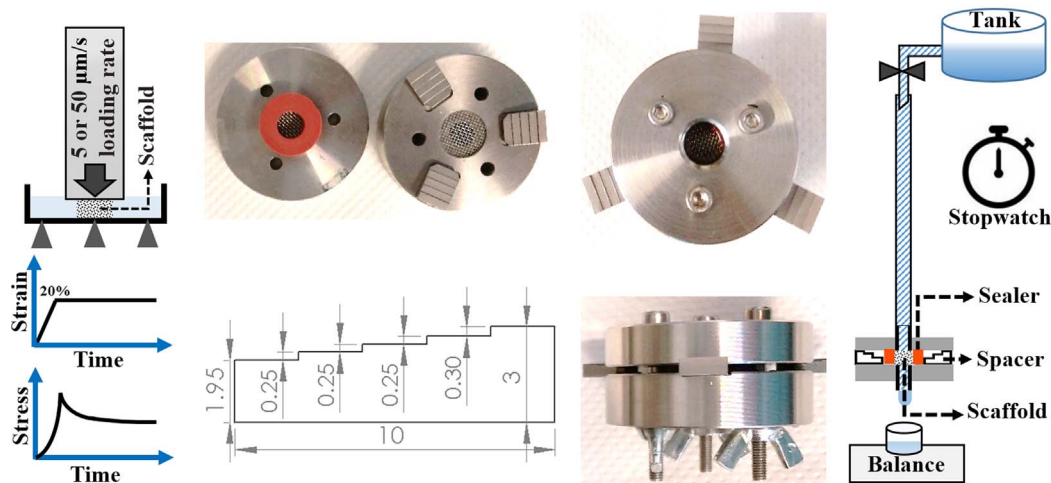


Fig. 1. Left: Schematic illustration of the stress relaxation test. Center: permeability chamber with stepwise spacer and the sealant. Right: schematics of the experimental setup to measure permeability of a scaffold under different compressive strains.

permeability values from confined compression experimental data based on the assumed model were not reliable anymore. In particular, Ateshian et al. (1997) has already discussed the limitation of indirect estimation of the permeability from creep or stress-relaxation experiments when finite deformation biphasic theory is used (Ateshian et al., 1997). They showed low sensitivity of the reaction force vs. time in confined compression (e.g. stress relaxation or creep tests) to large variations of the permeability function parameters. The difficulty of the indirect methods resides in the parameters estimation of the mathematical function for samples with different porosities, pore sizes, interconnectivity and viscoelastic properties, which indeed requires several experiments.

In this work, we present a general experimental technique to accurately characterize strain dependent permeability of tissue engineering scaffolds. In the proposed test rig, we designed a step-wise spacer for straightforward characterization of the permeability under different compressions while preserving initial and boundary conditions. As example, we applied this method to recently developed viscoelastic scaffolds presenting different average pore sizes subjected to different compression values.

2. Materials and methods

Macroporous scaffolds with different average pore sizes and crosslinking density (3 groups) of pHEMA-EGDMA were prepared by salt leaching method as reported elsewhere (Abdel-Sayed et al., 2014; Nasrollahzadeh and Pioletti, 2016). Each swelled scaffold was cut with a 8 mm diameter punch and the thickness was sized to 3 mm using a custom made cutting tool. The equilibrium Young modulus of hydrated scaffolds were determined through applying successive compressive strains (10%, 15% and 20%) with the Instron uniaxial testing machine (Instron E3000, Norwood, Massachusetts, USA) as described elsewhere (Scholten et al., 2011). Unconfined stress relaxation tests of 20% strain were also performed for fine and coarse pore size scaffolds at 50 $\mu\text{m/s}$ and 5 $\mu\text{m/s}$ compression rates as schematically depicted in Fig. 1-Left (See Supplemental data for detail).

Micro-computed tomography scans (Skyscan 1076, Bruker-microCT, Kontich, Belgium) of freeze-dried scaffolds ($n=3$) was used to calculate with CTAn software (Bruker-microCT) the average and the distribution of pore sizes in dried state. By measuring the bulk geometry of the scaffolds in hydrated as well as in dried states, the pores volumetric expansion (PVE) was evaluated. We then estimated the corresponding average pore size change as cubic root of PVE ($\sqrt[3]{\text{PVE}}$) (Offeddu et al., 2016). Finally, by multiplying this value to obtained results from μCT of dried scaffolds, the hydrated state characteristics were calculated (see Supplemental data). The porosity ϕ was determined by measuring the volume V and weighting the mass of the swelled m_{wet} and the dried scaffolds m_{dried} according to Eq. (1).

$$\phi = \frac{(m_{\text{wet}} - m_{\text{dried}})/\rho_{\text{water}}}{V} \times 100 \quad (1)$$

We modified a previously proposed set-up by O'Brien et al. (2007) for measuring strain dependent permeability of scaffolds. In the developed set-up as

illustrated in Fig. 1-Center, we used a step-wise spacer, which allows measurements of the permeability in different compressive strains (10%, 18%, 27%, and 35%) in one assembly.

Before measuring the permeability of scaffold, we needed to insure that our set-up is leakproof. For this, a thick cylindrical sealant (3.5 mm thickness, 7 mm internal and 15 mm outer diameter) was designed to guarantee the sample seal while maintaining a uniform deformation. It was made of a silicon rubber (Elastosil M4601, Wacker Chemie, Munich, Germany) and we obtained its equilibrium Young modulus by sequential compressive strains of 5%, 10%, 15% and 20% considering stress relaxation after each step (Scholten et al., 2011). The pre-strain resulting in required sealing force to prevent any leakage under the applied experimental conditions was evaluated using Hooke's law and Lamé's equations for thick walled cylinders.

To calculate the permeability, as schematically shown in Fig. 1-Right, after stress equilibrium, the flow rate was measured under constant water head condition using the weight of the flowing water passing the scaffold ($n=4-6$) and a stopwatch. The permeability k of the scaffold was calculated using Eq. (2) based on Darcy's law (Darcy et al., 1856; Pennella et al., 2013).

$$k = \frac{QL\mu}{A\rho gh} \quad (2)$$

where, μ represents the dynamic fluid viscosity (0.001 Pa s for water), A is the actual scaffold cross section ($\pi r^2 = 2.83 \times 10^{-5} \text{ m}^2$), L stands for the thickness of the sample (m), h is the constant fluid head (2.04 m), Q denotes the measured volumetric flow rate (m^3/s), ρ is the density of the fluid (1000 kg/m^3 for water) and g is the gravity constant (9.81 m/s^2). The thickness of sample decreases in Eq. (2) in case of different compressive strains corresponding to steps of the spacer (3.00, 2.70, 2.45, 2.20 and 1.95 mm, respectively).

3. Results

We developed different types of viscoelastic scaffold having elastic modulus (0.76–1.2 MPa) within the range of articular cartilage by tuning the crosslinker (Cr) percentage of the base material and pore size as reported in Table 1. The average pore size and porosity for the three types of scaffolds ranged from 162 to 271 μm and 63% to 68%. The obtained results from the stress relaxation tests (Fig. 2-Bottom right) revealed that the initial decay rate and relaxation time of transient stress were significantly different between fine and coarse pore size scaffolds (see Supplemental data for detail).

The compressive modulus of the rubber sealant was $1226 \pm 154 \text{ kPa}$ and, therefore, with any value higher than 5% pre-strain of the sealer, leakproof condition for permeability measurement was insured. Typical measurement times varied between 2 and 60 min (after reaching steady state condition) depending on the scaffold pore size and applied compression. Performed permeability measurements revealed significant decrease in the permeability as the

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