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Design and Fabrication of 3D-Plotted Polymeric Scaffolds in Functional Tissue Engineering

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Regenerating the load-bearing tissues requires 3D scaffolds that balance the temporary mechanical function with the biological requirements. In functional tissue engineering, designing scaffolds with biomimetic mechanical properties could promote tissue ingrowth since the cells are sensitive to their local mechanical environment. This work aims to design scaffolds that mimic the mechanical response of the biological tissues under physiological loading conditions. Poly(L-lactide) (PLLA) scaffolds with varying porosities and pore sizes were made by the 3D-plotting technique. The scaffolds were tested under unconfined ramp compression to compare their stress profile under load with that of bovine cartilage. A comparison between the material parameters estimated for the scaffolds and for the bovine cartilage based on the biphasic theory enabled the definition of an optimum window for the porosity and pore size of these constructs. Moreover, the finite element prediction for the stress distribution inside the scaffolds, surrounded by the host cartilaginous tissue, demonstrated a negligible perturbation of the stress field at the site of implantation. The finite element modeling tools in combination with the developed methodology for optimal porosity/pore size determination can be used to improve the design of biomimetic scaffolds. *POLYM. ENG. SCI.*, 47: 608–618, 2007. © 2007 Society of Plastics Engineers*

INTRODUCTION

Articular cartilage plays an essential role in freely moving joints because it provides a near-frictionless and low-wear bearing surface for the articulating bones and helps to absorb mechanical loads. It consists of a porous extracellular matrix (made up primarily of collagen fibrils and proteoglycan gel), interstitial fluid (water), and cells

(chondrocytes). The composition ratio is roughly 75% of fluid and 25% of solid matrix by total weight, and the exact composition depends greatly on location on the articular surface, depth, and age [1]. Because of its avascular nature, cartilage exhibits a very limited capacity to regenerate and to repair under injury or arthritic disease.

Current treatments either have limited success in terms of their efficiency or have unacceptable side effects. For example, the autologous chondrocyte implantation procedure lacks inter-patient consistency and the surgical procedure is technically challenging [2, 3]. Moreover, the reparative tissue produced after most cartilage repair techniques cannot withstand the demands required of an articular surface and quickly degenerates since most techniques are not able to produce hyaline cartilage [4]. Tissue engineering approach using 3D scaffolds is a novel alternative to conventional repair techniques. It consists of seeding highly porous biodegradable scaffolds with cells and growth factors in vitro, followed by culturing in a bioreactor to promote tissue growth. The scaffold degrades over time while leaving place for ingrowing tissues. Finally, once a certain level of tissue growth is achieved, the scaffold-tissue construct is surgically implanted into cartilage lesion in vivo. The use of bioresorbable matrices in the treatment of chondral defects holds promise since the regenerated hyaline cartilage shows full integration after several weeks [5].

The research on polymeric scaffold materials is mostly driven by the regulatory approved biodegradable and bioresorbable polymers, such as polyglycolide (PGA), polylactides (PLLA, PDLA), polycaprolactone (PCL), etc [6]. The design and fabrication of porous constructs based on these materials is critical for the success of tissue engineering. In general, scaffolds must satisfy the following requirements: (a) provide a space that will define the shape of the regenerating tissue, (b) provide temporary mechanical support during tissue regeneration, and (c) facilitate tissue ingrowth by allowing the inclusion of seeded cells and growth factors [7]. The second and third requirements imply conflicting design goals. While

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a dense scaffold is required for adequate mechanical properties, cell migration and nutrient transport are facilitated by a porous scaffold. On the other hand, the stiffness of the scaffold may alter the metabolic activity of the cells [8]. Therefore, these constructs should be designed to match the stiffness and strength of healthy tissues while maintaining an interconnected pore network and a reasonable overall porosity and pore size range [9].

The scaffold fabrication technique should be flexible to generate alternative scaffold architectures in order to allow biomimetic designs. A variety of techniques is currently being employed for fabrication of porous polymeric scaffolds, including phase separation [10, 11], gas foaming [12], porogen leaching [13], fiber bonding [14], emulsion freeze-drying [15], or a combination of these techniques [16–18]. While the conventional scaffold fabrication techniques can produce highly porous scaffolds, they have very limited control over scaffold architecture and pore interconnectivity. Therefore, the emerging solid free-form fabrication (SFF) techniques are becoming the method of choice for tissue engineering applications. Among these processes, the 3D-plotting technique has shown a great potential in producing reproducible 3D scaffolds featuring interconnected pores and controlled architecture [19]. In contrast to conventional rapid prototyping systems, which are mainly focused on fused deposition, the 3D-plotting technique can be applied to a much larger variety of synthetic and natural materials, including aqueous solutions and pastes [20]. This technique also allows prototyping at body temperature, especially of interest if living cells are incorporated into the plotting material. A comparison between the rapid prototyping techniques for tissue engineering applications can be found elsewhere [21].

Each tissue requires a specific scaffold design with a set of minimum biological and physical requirements [22]. Recent advances in both computational topology design and SFF fabrication have made it possible to design and fabricate scaffolds with controlled architectures, mimicking the mechanical properties of host tissues [7, 23]. However, most of these design tools require a complex computational algorithm when it comes to soft tissues. Hence, incorporating the viscoelastic or biphasic properties of soft tissues into the design variables could have a significant impact on the computational time of these design tools. Therefore, a computational approach with a reduced level of complexity, and some input from experimental data, could significantly improve the efficiency of the design process.

In this paper, we explore a new computational approach to designing scaffolds that could meet the requirements for the functional tissue engineering. This approach combines the identification of an optimum window for the porosity level and pore size of scaffolds, aiming to address both mechanical and physiological requirements, with a numerical tool that predicts the stress field for the scaffold-tissue constructs at the site of implantation. We focus on designing scaffolds that mimic the mechanical response of the host tissues, to minimize the perturbation

of the physiological stress fields after implantation. In regenerating the load-bearing tissues, this kind of design strategy could promote the tissue ingrowth since the cells are sensitive to their local mechanical environment [8]. Moreover, an implant that follows the deformation profile of the host tissue has a better chance of physiological integration because of reduced physical gap between the construct and the host, and improved migration of the host cells. Based on these hypotheses, this article focuses on the following specific objectives:

- Investigate the effect of scaffold architecture on its mechanical response under compressive loads.
- Determine optimal scaffold architectures to mimic the mechanical response of the cartilage under compression while providing maximum porosity to promote tissue ingrowth.
- Verify the perturbation of the mechanical environment (stress profiles) upon scaffold implantation inside host tissue using a finite element modeling tool.

To explore the pertinence of the computational approach, PLLA scaffolds with varying porosities and pore sizes were made by the 3D-plotting technique. The scaffolds were subsequently characterized under unconfined ramp compression, to define the optimum window for scaffold topology.

THEORETICAL BACKGROUND

Governing Equations

During joint movements, the load is distributed and friction is minimized by articular cartilage. This load-bearing capacity comes from the biphasic nature of the cartilage. Because of the relatively low permeability of the extracellular matrix, the interstitial fluid cannot easily escape from the tissue under the mechanical loading. Therefore, fluid pressurization has a major contribution to load support [24]. Mow et al. [25] developed a biphasic theory to describe the load-bearing characteristics of cartilage based on the smeared approach. According to this model, the solid matrix is assumed to be linearly elastic or hyperelastic, and the viscous dissipation is a result of the frictional drag forces, which are directly proportional to the relative velocity of fluid flow to the solid matrix. The biphasic model is applicable as well to scaffolds because of their porous structure, filled with a fluid in either a bioreactor or a physiological environment.

According to the biphasic model, cartilage has a porous structure and the flow of fluid through the tissue is governed by Darcy's law:

$$u = -\frac{k}{\mu} \nabla p \quad (1)$$

where k denotes the intrinsic permeability of the porous structure (in m^2), μ is the fluid viscosity, p is the pressure,

and u is the fluid velocity. Darcy's law describes the flow in porous media with the pressure gradient as the only driving force and assumes that the transport of momentum by shear stresses in the fluid is negligible. Moreover, it is based on the homogenization of the solid and fluid media into one single medium and does not require a detailed geometrical description of the pore structure. The balance of mass for the fluid/solid constituents and for the mixture can be represented by the following equations [26]:

$$\phi^s + \phi^f = 1 \quad (2)$$

$$\nabla \cdot (\phi^s v^s + \phi^f v^f) = 0 \quad (3)$$

where ϕ^s and ϕ^f are the volume fractions of solid and fluid, respectively, and v^s and v^f are the solid and fluid velocities. In this model, the material incompressibility for each constituent is respected while allowing the overall volume to change through the changes in the volume fraction of the fluid. The momentum equations for the biphasic theory take the following forms [25–27]:

$$\nabla \cdot \sigma^s + \pi^s = 0 \quad (4)$$

$$\nabla \cdot \sigma^f + \pi^f = 0. \quad (5)$$

In these equations, σ^s is the Cauchy stress tensor, σ^f is the fluid stress, and π^s and π^f are the diffusive momentum exchange, representing the local interaction forces between the solid and fluid constituents, given by:

$$\pi^s = -\pi^f = p\nabla\phi^s + K(v^f - v^s) \quad (6)$$

where K is a second order tensor that measures the frictional resistance against fluid flow through the solid matrix (diffusive drag). Due to the low permeability of biological soft tissues, inertial terms and external body forces are considered negligible at physiological loading conditions when compared with the diffusive drag forces [26].

FIG. 1. Schematics of the applied ramp displacement and the corresponding stress-relaxation under unconfined compression test.

Analytical Formulation

To estimate the biphasic parameters of the scaffolds, the analytical solution of the biphasic model under unconfined compression configuration was fitted to the transient stress response of the scaffolds under a compressive ramp displacement. The unconfined compression test, schematically shown in Fig. 1, consists of imposing a displacement to a thin cylindrical disk located between two rigid impermeable parallel quasifrictionless platens. The sample is free to expand radially, and the pore fluid exudation occurs on the sides of the sample. In a typical cartilage, once a desired level of strain is reached, the sample exhibits stress-relaxation until equilibrium is attained (Fig. 1). At equilibrium, no fluid flow exists and the entire load must therefore be borne by the solid matrix, thus eliminating the fluid-dependent viscoelasticity effects. According to Cohen et al. [28], the load intensity response to a ramp displacement at a constant strain rate of $\dot{\epsilon}_0$, imposed until time t_0 , is given by:

$$f(t) = E_3 \dot{\epsilon}_0 t + E_1 \frac{\dot{\epsilon}_0 a^2}{C_{11}k} \Delta_3 \left\{ \frac{1}{8} - \sum_{n=1}^{\infty} \frac{\exp(-\alpha_n^2 C_{11} K t / a^2)}{\alpha_n^2 [\Delta_2^2 \alpha_n^2 - \Delta_1 / (1 + v_{21})]} \right\} \quad \text{for } 0 < t < t_0 \quad (7)$$

$$f(t) = E_3 \dot{\epsilon}_0 t - E_1 \frac{\dot{\epsilon}_0 a^2}{C_{11}k} \Delta_3 \left\{ \sum_{n=1}^{\infty} \frac{\exp(-\alpha_n^2 C_{11} K t / a^2) - \exp(-\alpha_n^2 C_{11} K (t - t_0) / a^2)}{\alpha_n^2 [\Delta_2^2 \alpha_n^2 - \Delta_1 / (1 + v_{21})]} \right\} \quad \text{for } t > t_0 \quad (8)$$

where E_1 and E_3 are the Young's moduli, v_{21} and v_{31} are the Poisson's ratios, k is the Darcy's permeability that depends on the pore structure and pore fluid [in $\text{m}^4/(\text{N s})$], a is the radius of the sample, and

$$\Delta_1 \equiv 1 - v_{21} - 2v_{31}^2 E_1 / E_3 \quad (9)$$

$$\Delta_2 \equiv (1 - v_{31}^2 E_1 / E_3) / (1 + v_{21}) \quad (10)$$

$$\Delta_3 \equiv (1 - 2v_{31}^2) \Delta_2 / \Delta_1 \quad (11)$$

$$C_{11} = E_1 (1 - v_{31}^2 E_1 / E_3) / [(1 + v_{21}) \Delta_1] \quad (12)$$

and α_n are the roots of the transcendental equation:

$$J_1(x) - \left(\frac{1 - v_{31}^2 E_1 / E_3}{1 - v_{21} - 2v_{31}^2 E_1 / E_3} \right) x J_0(x) = 0 \quad (13)$$

in which J_0 and J_1 are Bessel functions of the first kind. It is reported in the literature that the out-of-plane Poisson's ratio (ν_{31}) can be set to zero without compromising the accuracy of the model [29, 30]. This is based on the observation that the equilibrium stress in the axial direction is the same in confined and unconfined compression, which implies that the radial stress is zero in confined compression. It should be noted that for an isotropic material, $E_1 = E_3$, and $C_{11} = \lambda_s + 2\mu_s = H_A$, where λ_s and μ_s are the Lamé constants, and H_A is the aggregate modulus. Therefore, the stress response reduces to the analytical solution for purely isotropic biphasic material developed by Armstrong et al. [29].

Finite Element Formulation at Equilibrium

Cartilage exhibits stress-relaxation under load until equilibrium is attained. As it was mentioned earlier, no fluid flow exists at equilibrium and the entire load is borne by the solid matrix. This eliminates the fluid-dependent viscoelasticity effects and leads to a linear relationship between the compressive stress and strain at equilibrium. This has been confirmed experimentally up to ~20% strain level [24]. Thus a hyperelastic constitutive model was used to predict the stress field at equilibrium.

In continuum mechanics, the large deformation formulation of incompressible bodies is solved using the principle of stationary potential energy [31–33]. The total energy Π of a deformed body submitted to external loads tends to be minimal with regards to the displacement and pressure fields u and p :

$$\delta\Pi(u, p) = \delta\Pi_{\text{int}}(u, p) - \delta W_{\text{ext}}(u) = 0 \quad (14)$$

where W_{ext} is the external work and Π_{int} is the strain energy defined by the constitutive model. The strain energy function is separated into isochoric and isostatic strain energies, Π_d and Π_p :

$$\Pi_{\text{int}} = \Pi_d(I_1^*, I_2^*) + \Pi_p(I_3) \quad (15)$$

where I_1^* and I_2^* are the first isochoric invariants of the Cauchy strain tensor and I_3 is the third invariant. The isochoric invariants are defined by:

$$I_1^* = I_1 I_3^{-1/3} \quad (16)$$

$$I_2^* = I_2 I_3^{-2/3} \quad (17)$$

with I_1 and I_2 being the two first invariants of the Cauchy strain tensor. The variation of the strain energy is expressed as a function of the deviatoric second Piola-Kirchhoff stress tensor S^d and the Green–Lagrange strain tensor E :

$$\delta\Pi_{\text{int}} = \delta \int_{\Omega_0} S_{ij}^d \delta E_{ij} d\Omega_0 + \delta\Pi_p(I_3) \quad (18)$$

where Ω_0 is the volume of the undeformed body. The incompressibility constraint is solved using the augmented Lagrange method where the penalty term is expressed as:

$$\Pi_p(I_3) = \frac{\kappa}{2} (I_3^{1/2} - 1)^2. \quad (19)$$

The major advantage of this method lies in its capacity for solving the ill-conditioned problem of the penalty method using lower values of the penalty constant κ , while providing a solution that exactly addresses the incompressibility constraint [34]. The resulting system can be solved by decoupling the pressure and displacement fields [35, 36].

The hyperelastic material model used in this work is the two-parameter Mooney–Rivlin constitutive equation, given by the following strain energy function [37]:

$$\Pi_d = c_1(I_1^* - 3) + c_2(I_2^* - 3). \quad (20)$$

This model reduces to the Hookean model by substituting $c_2 = 0$ and $c_1 = E/6$, where E is the Young's modulus.

MATERIALS AND METHODS

Preparation of the Plotting Material

The plotting material was prepared by dissolving PLLA with a molecular weight of 220 kDa (Biomater, Germany) in methyl ethyl ketone (MEK). The dissolution process was on average 1–3 days for grinded PLLA granules, and 3–5 days for whole polymer granules. The mixture was stirred thoroughly at least once a day to speed up the dissolution process. A negligible amount of solvent was added each time after stirring to prevent drying of the partially dissolved resin. The optimal concentration of the polymer in solvent was determined based on the viscosity constraints of the 3D plotter while targeting optimal syringe deposition. It was found that a concentration of 0.4 g PLLA/0.6 g of solvent provided an adequate viscosity without compromising smooth deposition of the paste (see Rheological Measurements).

Scaffold Fabrication

The scaffolds were fabricated using a bioplotter from EnvisionTec, which is essentially an XYZ 3D plotter as described by Landers et al. [20]. The apparatus has a built-in controller for precise material deposition, and is integrated with PrimCam v2.96 software. The polymer solution was transferred to the plotting cartridges with 250- μm dispensing needle tips and was dispensed layer by layer. Starting from the bottom layer, each newly formed layer adhered to the previous and was perpendicular to it, thus forming a 0°/90° strand structure (Fig. 2a). It is reported that this configuration is potentially the most

FIG. 2. (a) Schematics of the 3D-plotting technique; (b) adjustable plotting parameters of the 3D plotter.

effective in mimicking the biomechanical behavior of bovine cartilage [19]. The strands within each layer were laid with an offset with respect to the previous layers in order to form a staggered pattern (Fig. 2b). A CAD file specifying the geometry of the scaffold was used as input to produce the physical model by the apparatus. Bricks of $20 \text{ mm} \times 20 \text{ mm}$ wide and 4 mm thick were fabricated during this process. The 3D-plotted bricks were then air-dried for 24 h and vacuum-dried for 48 h to allow complete evaporation of the solvent. Subsequently, disks of 6 mm diameter were punched out of the bricks using surgical biopsy punches for porosity measurements and mechanical testing.

Determination of the Plotting Parameters

Experiments were performed to determine the influence of the plotting parameters on scaffold porosity and mechanical properties. The primary parameters were the internal diameter of the dispensing needle, dispensing speed, layer thickness, strand diameter, and the distance between the strands, representing the pore size of the constructs (see Fig. 2b). Needles with larger tip diameters ($>250 \mu\text{m}$) generally produced scaffolds with thicker strands, which had lower porosities and higher Young's Modulus. Therefore, needles with a tip diameter of $250 \mu\text{m}$ were used in this study. On the other hand, increasing the layer thickness h (center-to-center distance between the successive 3D-plotted strands) compromised the adhesion of the strands between two successive layers, and as a consequence the integrity of the scaffold. It was found that a layer thickness below $140 \mu\text{m}$ provided adequate adhesion between the layers for a needle tip diameter of $250 \mu\text{m}$.

After fabrication and solvent evaporation, the 3D scaffolds were cut vertically across the middle with a sharp razor, and their cross sections were examined and photographed using an optical microscope. The mean strand diameter for each scaffold was measured from

these digitally captured images, and the dependence of the strand diameter on the dispensing speed was analyzed. Increasing the dispensing speed reduced the strand diameter and directly influenced the porosity of the scaffolds, as it can be seen in Fig. 3. This figure also shows that the strand diameter is lower than the diameter of the dispensing needle ($250 \mu\text{m}$), clearly because of the stretching effect of the dispensing arm. The distance between the strands was kept constant for these experiments ($300 \mu\text{m}$). On the other hand, Fig. 4 shows that increasing the distance between the strands increased the scaffold porosity while reducing the initial Young's modulus for the constructs (see Mechanical Characterization). The strand diameter was $220 \mu\text{m}$ in these experiments. Table 1 presents a typical combination of the plotting parameters that produced scaffolds with porosities of 65, 75, and 85% (v/v), featuring low strand diameters ($\leq 200 \mu\text{m}$) and relatively low Young's modulus. The machine-set values are also compared with the physically-produced strand

FIG. 3. Effect of dispensing speed on scaffold porosity and strand diameter.

FIG. 4. Effect of the distance between the strands (pore size) on scaffold porosity and initial Young's modulus.

dimensions for these scaffolds. The emphasis in this paper is placed on these three scaffold topologies.

Rheological Measurements

The linear viscoelastic measurements were conducted on the plotting material (PLLA/MEK solution) using an ARES/Rheometrics Rheometer at room temperature. Due to the volatile nature of the solution, the multiwave single-point testing procedure was used. This procedure is based on the Boltzmann superposition principal [38] and permits rapid frequency sweep tests by creating multiple sinusoidal waves from a single frequency [39]. The storage and loss moduli curves as function of frequency, $G'(\omega)$ and $G''(\omega)$, as well as the complex viscosity curve, $\eta^*(\omega)$, were obtained for three replicates.

Porosity Measurements

The true volume of each scaffold was calculated based on the polymer density ($\rho_{PLLA} = 1.2 \text{ g/cm}^3$) and scaffold mass (m) using $V_t = m/\rho$. The scaffold porosity (in vol%) was calculated using the following equation:

$$\phi = \frac{(V_a - V_t)}{V_a} \times 100 \quad (21)$$

where V_a is the apparent volume of the scaffold (in cm^3) estimated based on the geometry of each disk (thickness and diameter). The scaffold density at different porosity levels was also calculated using $\rho = \rho_{PLLA}(1-\phi)$, and listed in Table 1.

Mechanical Characterization

Unconfined compression tests were conducted on both porous scaffolds and nonporous PLLA samples using ELF series Enduratec apparatus in a saline bath (0.009 g/cm^3 salt concentration) at 37°C . Melt-compressed, nonporous samples of 1 mm thick were prepared using single granules of PLLA. Subsequently, disks of 3 mm diameter were punched out for the tests. Prior to mechanical testing, all samples were conditioned for 24 h inside the saline solution (37°C), allowing the saturation of the constructs with the solution (swelling).

Porous Scaffolds. The tests were conducted under displacement-controlled loading. Three series of successive ramp strains (3% each for a total of 9% strain) were applied at a displacement rate of $0.115 \mu\text{m/s}$, and the samples were allowed to relax for 30 min after each ramp. Three samples were tested at each porosity level (65, 75, and 85%). The slope of the first compressive ramp, representing the initial Young's modulus, was estimated for all samples.

Nonporous Disks. The tests were conducted under force-controlled loading. A single ramp strain was applied at a displacement rate of $0.115 \mu\text{m/s}$, and the samples were allowed to relax for 7 min once the force reached 7 N (equivalent to 1 MPa compressive stress). This upper limit for the force was chosen based on the highest compressive stress observed for the porous scaffolds under displacement-controlled loading ($<1 \text{ MPa}$, see Results and Discussion). The initial Young's modulus was estimated for three replicates.

Biphasic Parameter Estimation

The permeability and equilibrium modulus were estimated based on both purely isotropic and transversely isotropic biphasic models [28, 29]. The biphasic parameters

TABLE 1. Summary of the plotting parameters (machine-set vs. physical) and calculated densities for the scaffolds at different porosity levels.

Scaffold	Porosity (%)	Density (g/cm^3)	Dispensing speed ($\mu\text{m/s}$)	D_b^a (μm)	L (μm)		h (μm)	
					Machine-set	Physical	Machine-set	Physical
A	65	0.42	98	200	300	280	130	120
B	75	0.30	165	150	200	180	130	120
C	85	0.18	165	150	400	370	140	130

^aNeedle inner diameter: $250 \mu\text{m}$.

FIG. 5. Comparison between (a) purely isotropic biphasic, and (b) transversely isotropic biphasic models fitted to typical single ramp compression-stress relaxation data.

for cartilage were estimated using the unconfined compression data for bovine cartilage from literature [30], which was characterized under similar testing conditions. A comparison was made between the load intensity responses predicted by the two models for the analysis of the quality of the fits obtained by each model. It should be noted that for simplicity reasons, the moduli for the transversely isotropic case (E_3 and C_{11} in Eqs. 7–13) are represented by E_z and H_A , respectively.

Figure 5 shows a comparison between the fits obtained based on purely isotropic and transversely isotropic biphasic models. While the quality of the fits were comparable at small compressive strains (not shown), at large deformations (third ramp in particular) the purely isotropic biphasic model did not provide a good fit (see Fig. 5a). Therefore, in this paper the emphasis is placed on the transversely isotropic biphasic model.

Numerical Simulations

IMI's finite element modeling software FormSim© [36] was used to predict the stress distribution at equilibrium under a compressive load for the scaffolds (65 and 85% porosities), which were surrounded by cartilaginous host tissues. For the bovine cartilage and for the scaffolds, the respective compressive Young's moduli at equilibrium (E_z) were used in the simulations (see Eq. 20). The finite-element mesh was composed of separate meshes for the scaffold and the cartilage. An eight-node brick element was used to create the finite element mesh of the scaffold (2604 elements) and that of cartilage (3876 elements).

The virtual contact between the scaffold and the tissue at the interface was predicted based on the multibody contact algorithm [40].

RESULTS AND DISCUSSION

Experimental Analyses

Figure 6a and b show the storage and loss moduli, and complex viscosity curves as a function of frequency, respectively. According to the Maxwell viscoelastic model [41], the inverse of the crossover frequency for the storage and loss moduli curves provides an estimate for the relaxation time of the plotting material ($\tau = 0.89$ s, Fig. 6a). The complex viscosity of the solution reveals a shear-thinning behavior, as expected for a polymeric solution. The shear-thinning index for the material is estimated based on the power-law model [38], using the slope of the complex viscosity curve ($n = 0.57$, Fig. 6b). These rheological data could provide a basis for comparison when the plotting material is prepared using other biopolymers, or using different molecular weights of the same biopolymer. Once the desired viscosity range is achieved for a given polymeric solution, minimal adjustments in machine settings would be required to achieve a desired strand diameter.

The flexibility of the 3D-plotting technique to alternative design parameters allowed the production of scaffolds at a wide range of porosity levels and pore sizes, leading to different mechanical properties (modulus and permeability). Figure 7a and b show the SEM micrographs of the scaffold with a porosity of 85%, top and cross-sectional views, respectively. The photomicrograph of the disk-shaped scaffold is shown in Fig. 7c. It can be seen that the scaffold has a uniform pattern throughout its layers, leading to a homogeneous architecture. These images also reveal a very good consistency between the machine settings and the physically-produced strand dimensions (see Table 1). The noticed small discrepancy is attributed to the material shrinkage after solvent evaporation.

FIG. 6. (a) Storage and loss moduli, and (b) complex viscosity curve as function of frequency. The relaxation time (τ) and shear-thinning index (n) for the plotting material are estimated in these figures.

FIG. 7. (a,b) SEM micrographs showing the top (a) and the cross-sectional view (b) of 3D-plotted scaffold at a porosity of 85% ($\times 100$); (c) photomicrograph of the disk-shaped scaffold.

Figure 8a compares the measured stress levels corresponding to successive strain ramps in 3D-plotted scaffolds and in the bovine cartilage from literature [30], tested under similar conditions. It can be observed that the level of stress in the 3D-plotted samples with 85% porosity is comparable to that of bovine cartilage. This is primarily attributed to the fact that the articular cartilage is composed of up to 85% water. Therefore, a tissue-engineering scaffold aimed for cartilage regeneration requires a high porosity level to mimic the mechanical response of cartilage under physiological loading while enhancing the tissue growth through its highly porous structure. These results also indicate the viscoelastic nature of the porous constructs, saturated with the saline solution. The corresponding results for the nonporous disks are given in Fig. 8b. The lack of stress relaxation phenomenon for these samples demonstrates the elastic nature of the PLLA at solid state. Therefore, the fluid-independent viscoelasticity, coming from the polymer matrix itself, can be excluded based on these observations.

Figure 9a compares the estimated biphasic parameters for the scaffolds with those of bovine cartilage [30]. As expected, both the aggregate and the equilibrium Young's modulus decrease and the permeability increases as the scaffold porosity increases. The magnified scale of the estimated parameters at the highest porosity level, given

in Fig. 9b, indicates that the 3D-plotted constructs at 85% porosity mimic both the equilibrium Young's modulus (E_z) and the permeability (k) of the bovine cartilage while showing a discrepancy in terms of the aggregate modulus (H_A). To avoid this discrepancy, reducing the strand diameter would be necessary, which would require reducing the needle-tip diameter by the manufacturer. The estimated parameters of the biphasic model (third ramp, 9% compressive strain) as well as the initial Young's modulus (first ramp) for the scaffolds at 85% porosity are given in Table 2.

The shape of the stress relaxation curve could provide useful information on the level of viscoelasticity of the scaffold samples. The 3D-plotting technique leads to viscoelastic constructs with controlled pore structures. Moreover, the strand layout in the 3D-plotting technique offers anisotropic characteristics to these constructs. However, this technique has a limitation on the minimum achievable strand diameter ($D_b > 140 \mu\text{m}$).

At this point, one can establish an optimum window for the scaffold architecture to allow mimicking the mechanical response of the bovine cartilage. Focusing on the third ramp (9% compressive strain), for which the model parameters were estimated, the optimal range of the porosity level is estimated in Fig. 10. In this figure, the dashed lines represent the estimated biphasic parameters

FIG. 8. (a) Stress relaxation under successive ramps compression for the scaffolds at different porosity levels compared with that of bovine cartilage [30] (displacement-controlled loading); (b) corresponding results obtained at a single ramp for the nonporous disks (force-controlled loading).

FIG. 9. (a) Estimated parameters of the transversely isotropic biphasic model for the scaffolds at different porosity levels and for bovine cartilage [30]; (b) magnified scale for the scaffold at 85% porosity.

(E_z , H_A , and k) as a function of scaffold porosity. The stars indicate the crossover points where the scaffolds meet the biphasic requirements of the bovine cartilage. The optimal porosity of the 3D-plotted scaffolds falls between 84 and 89 vol%, corresponding to a pore size range of 350–450 μm (see Table 1). The summary of the optimal architectural parameters is shown in Table 3.

Numerical Simulations

Scaffolds used in literature for soft tissue repair generally have much lower compressive properties compared with the normal cartilage. The compressive modulus of the typical scaffolds made of alginate and agarose [42, 43] are usually between 10 and 100 times lower than that of bovine cartilage [30, 44, 45]. Since the cells are sensitive to their local mechanical environment, this property mismatch has the potential of affecting the level of tissue ingrowth.

The finite element software developed at IMI was used to predict the stress distribution in the scaffolds and in the bovine cartilage at equilibrium, under simulated physiological conditions. The modulus at equilibrium (E_z) was used in the simulations. The finite element mesh for the scaffold and for the cartilage is presented in Fig. 11a. Figure 11b shows the stress field at the site of implantation for the 3D-plotted scaffolds (65 and 85% porosity) surrounded by the cartilaginous tissue, under 9% compressive strain. The stress perturbation is not pronounced for the scaffold at 85% porosity. Based on these results, the

designed 3D-plotted constructs at 85% porosity meet the mechanical requirements of the cartilaginous tissue.

Due to the variations in the collagen fibril orientation and content, articular cartilage has nonuniform properties and composition throughout its thickness. The cartilage layers become progressively stiffer approaching the calcified region adjacent to the subchondral bone. Therefore, the property mismatch between the scaffold and the tissue can be reduced by designing scaffolds featuring similar variation in properties throughout their thicknesses. This can be done by tailoring pore size and porosity distribution to the variation in modulus and permeability of normal cartilage. The orientation of the strands in the successive 3D-plotted layers can be tailored to the structure of host tissue in order to create anisotropic structures mimicking articular cartilage. The 3D-plotting technique allows the fabrication of scaffolds with such variations in properties, thus enabling biomimetic designs. This design strategy will be the subject of our future work. The ionic environment present in cartilage is another design criterion that could be taken into consideration in the future, and can be addressed by using conductive polymers as scaffold materials [46].

TABLE 2. Summary of the estimated transversely isotropic biphasic parameters (H_A , E_z , and k : third ramp) and the initial Young's modulus (E : slope of the first ramp) for the scaffolds at 65 and 85% porosity and for the bovine cartilage [30].

Parameter	Scaffold (65%)	Scaffold (85%)	Bovine cartilage
H_A (MPa)	65.53 ± 8.55	4.33 ± 0.69	1.23
E_z (MPa)	5.77 ± 1.30	0.96 ± 0.29	1.09
k ($\text{e}^{-15} \text{ m}^4/(\text{N s})$)	0.06 ± 0.01	0.63 ± 0.08	0.87
E^a (MPa)	13.06 ± 1.15	1.06 ± 0.34	2.70

Poisson's ratio $\nu_{21} = 0.35$ was assumed ($\nu_{31} = 0$).

^aYoung's modulus for nonporous PLLA disks: 50.24 ± 4.51 MPa.

FIG. 10. Determination of the optimum window for the scaffold-plotting parameters (third ramp: 9% compressive strain). Dashed lines are the estimated biphasic parameters for the scaffolds as function of porosity. The stars indicate the crossover points where the scaffolds meet the biphasic requirements of bovine cartilage.

TABLE 3. Summary of the estimated optimal architectural parameters for the scaffolds based on the transversely isotropic biphasic model.

Parameter	Optimal range
Porosity (%)	84–89
Pore size (μm)	350–450
Strand diameter (μm)	100–150

One of the major causes of implant rejection is avascular necrosis of the implant core. This is generally attributed to the increased distance from blood vessels and poor nutrient transport in host tissue [47, 48]. Moreover, a rapid tissue formation on the outer edge of the scaffold can restrict cell penetration and nutrient exchange to the scaffold center [49]. In this work, we designed scaffolds with fully interconnected pores and a high level of porosity that could help to promote nutrient transport. Anisotropic scaffold architectures featuring a distribution of micro- and macropores will be used in our future studies to enhance cell penetration and reduce the risk of necrotic cores [49].

Another challenge in replacing a diseased cartilage is to ensure that the regenerated tissue is of the right type. Although dynamic viscoelastic measurements can be used to differentiate the two types of cartilage (hyaline cartilage and fibrocartilage) [50], the parameters leading to the desired state of tissue regeneration are yet to be explored. These parameters include the optimal composition of scaffolds and the microenvironment supplied by these constructs [51]. Therefore, in our future studies we will aim to discover the optimal conditions that enable articular cartilage repair and that lead to minimal side effects on the state of the surrounding tissues at the site of implantation.

CONCLUSION

This work presented a new computational approach to designing scaffolds that meets both mechanical and biological requirements for functional tissue engineering. This approach targeted the biphasic properties of bovine cartilage in designing the scaffolds aimed for cartilage regeneration. PLLA scaffolds were fabricated by the 3D-

plotting technique and mechanically tested under successive unconfined ramp compression to estimate their biphasic parameters. An optimum window for the porosity level and pore size of the scaffolds were then identified to mimic the mechanical response of bovine cartilage under compressive loads. Lastly, a comparison was made between the predicted stress distribution at equilibrium in the scaffolds and in host tissue, using the finite element modeling software developed at our institute. These results demonstrated that the designed 3D-plotted constructs meet the mechanical requirements of the load-bearing tissues.

The subject of our future work is to design hybrid osteochondral scaffolds, which will maintain the mechanical and physiological requirements of host cartilage/bone tissues during their structural evolution. We believe this will be a critical step towards ensuring more effective *in vivo* implantation and improved biological integration. The variation of properties throughout the thickness of the scaffold will more closely imitate true articular cartilage structure. This will also improve physiological integration through the bone fixation, while reducing the risk of necrosis. Our modeling tool will be used to predict the biphasic stress distribution throughout the osteochondral scaffold-tissue constructs under physiological conditions and its coupling with scaffold degradation and tissue growth kinetics. Our current work focused on macroscale modeling of scaffold-tissue constructs based on the smeared approach. A multiscale-modeling approach accounting for microscale features of the scaffold and for cell biomechanics will be considered in our future studies.

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FIG. 11. (a) Finite element mesh of a scaffold, surrounded by a typical cartilage; (b) predicted solid-stress distribution at equilibrium under 9% unconfined compression strain for the scaffolds (65 and 85% porosity) and surrounding cartilage. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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