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SCAFFOLD GEOMETRY INFLUENCES THE MECHANICAL PROPERTIES OF TISSUE ENGINEERED CARTILAGE

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Abstract. A common problem in tissue engineered cartilages is the production of tissues with stiffer peripheries and softer cores due to insufficient nutrient supply. The introduction of channels in the scaffolds promotes more homogeneous nutrient supply. Our aim was to simulate the impact of the introduction of a macrochannel in cylindrical or parallelepipedic scaffolds on nutrient supply, cell density, extracellular matrix (ECM) production and remodeling of the biphasic mechanical properties. A finite element tool was developed in Abaqus and a static culture period of 72 hours was simulated. The channeled constructs had more homogeneous nutrient distributions, with an up to 136-fold increase in minimum glucose concentrations and 220-fold increase in minimum oxygen concentrations. A 9% increase in global cell density and 50% increase in global glycosaminoglycans and collagen concentrations was achieved for channeled constructs. However, the low values of ECM concentrations after 72h resulted in a relative increase of 4% in Young's modulus and 2% decrease in permeability. The cylindrical constructs are more favorable geometries due to more homogeneous nutrient diffusion. The introduction of a single macrochannel is important to obtain more homogeneous distributions. However, longer culture periods are required for a remarkable impact of the macrochannel on cartilage remodeling.

1 INTRODUCTION

Tissue engineered (TE) cartilage has been proposed as a prospective treatment for osteoarthritis [1]. TE cartilage is obtained by seeding chondrocytes on a porous scaffold comprised of a biomaterial that is able to provide a favourable environment to maintain the differentiated phenotype of chondrocytes, as well as to enable to production of extracellular matrix (ECM). TE cartilage constructs cultured on bioreactors are often small cylinders subjected to static of dynamic regimes. One of the limitations of this approach is that the cultured constructs have stiffer peripheries and softer cores. This is related to insufficient nutrient supply to the centre of the construct, combined with high nutrient uptake and matrix

production in the periphery, hindering the diffusive transport of nutrients to the centre [2-4]. These inhomogeneous tissues have inferior mechanical properties and ECM distributions when compared to the native cartilage, leading to implant failure [3]. In order to increase nutrient transport to the core of the constructs and homogenize the engineered tissues, the impact of the introduction of channels in the centre of the scaffolds has been assessed. The introduction of several narrow microchannels (diameter below 1 mm) on engineered cartilage, under dynamic culture, have provided more uniform distributions of ECM components when compared with solid scaffolds. However, this is accompanied by an increased release of GAGs to the culture medium [2]. These narrow microchannels also become filled with matrix after a few weeks in culture, impairing the desired effect of decreasing the nutrients' diffusion path length [3]. A study that evaluated the impact of macrochannels in the scaffolds has concluded that the introduction of those larger channels increased the mechanical properties of the channelled constructs in comparison with the solid ones, with a more uniform collagen fibrillar network as well. The channels remained open for several weeks, having a positive impact in nutrient diffusion after a significant amount of ECM has already been formed [3]. Our goal with this work was to simulate the impact of the introduction of a macrochannel in scaffolds with different geometries on the biphasic mechanical properties. This was achieved by implementing a finite element (FE) tool that simulated the transport and consumption of glucose and oxygen on the scaffold and its impact on cell proliferation, synthesis of ECM and the remodelling of the elastic Young's modulus (E) and the hydraulic permeability (k).

2 METHODS

2.1 Model description

The total stress in the tissue is described by the biphasic model, with a linear elastic solid matrix contribution and a pore pressure (fluid stress) contribution as reported elsewhere [5]. The material is described by the initial Young's modulus (E_0), the Poisson's ratio (υ), the initial permeability (k_0) and the initial void ratio (e_0). The permeability, for the purposes of this work, was assumed to be initially isotropic and constant. The initial mechanical parameters used are listed in Table 1.

The transport of nutrients in the constructs in governed by the reaction-diffusion equation. The reactive term describing nutrient uptake to the cells is given by the Michaelis-Menten kinetics. The diffusion coefficient in the tissue is dependent on the void ratio and is a fraction of the diffusion coefficient of the solute in water following the Mackie-Mears relationship [4]. Cell density is modified by migration, proliferation and death. The proliferation rate is modulated by the glucose concentration under the Contois kinetics, which accounts for cell density saturation [6]. The death rate was assumed as constant [7]. Extracellular matrix (ECM) remodelling is modelled by the concentration of a limiting solute under a logistic growth model [8]. Based on previous experimental work, the limiting solute for GAGs is oxygen [8], while glucose was chosen as the limiting solute for collagen [9]. Finally, we simulated the influence of the concentration of GAGs and COL on the remodelling of two important biphasic mechanical properties, the Young's modulus (E) and the permeability (k). Simple relationships adapted from [10] were employed. All the parameters necessary for the biosynthetic equations were taken from the literature.

Table 1: Values of the model parameters

Parameter	Value	Reference
E_0	40 kPa	This work
υ_0	0.35	This work
k_0	5×10^{-12} $m^4.N^{-1}.s^{-1}$	[1]
e_0	4.0	[1]

2.2 Model implementation

Five different scaffold geometries were modeled in Abaqus 6.12: a solid cylinder with 8mm diameter and 5 mm height; hollow cylinder with 8mm outer diameter, 2 mm inner diameter and 5 mm height; solid parallelepiped with 8mm side and 5 mm height; hollow parallelepiped with a 2mm diameter cylindrical channel and a hollow parallelepiped with a 2mm side parallelepipedic channel. Each scaffold was meshed with approximately 5000 pore pressure-stress-temperature elements (C3D8PT).

Due to the analogy between mass and heat transport, in order to use the built-in procedures and element types available as much as possible, the nutrient transport, cell density and ECM synthesis equations were solved with temperature degrees of freedom. User-defined subroutines implemented in Fortran allowed to include the reactive terms in the equations and to update the mechanical properties of the material.

A static culture period was simulated for 72 hours for each scaffold. Boundary conditions for glucose and oxygen are defined at the lateral surfaces in contact with fluid with values of 7.769 $mol.m^{-3}$ and 0.192 $mol.m^{-3}$ respectively. The initial oxygen concentration is uniform and the same as the boundary value. We assumed that no ECM existed in the constructs at the start of the simulation. The system was solved sequentially and values of the previous simulations needed to solve the equation of the current parameter are entered as field variables.

3 RESULTS

3.1 Glucose and oxygen

The equilibrium between nutrient diffusion and uptake by cells is achieved after approximately 7 hours of culture for glucose and 2 hours for oxygen under the prescribed initial and boundary conditions. The steady-state profiles for the two solutes on the five simulated geometries are shown in Figure 3.

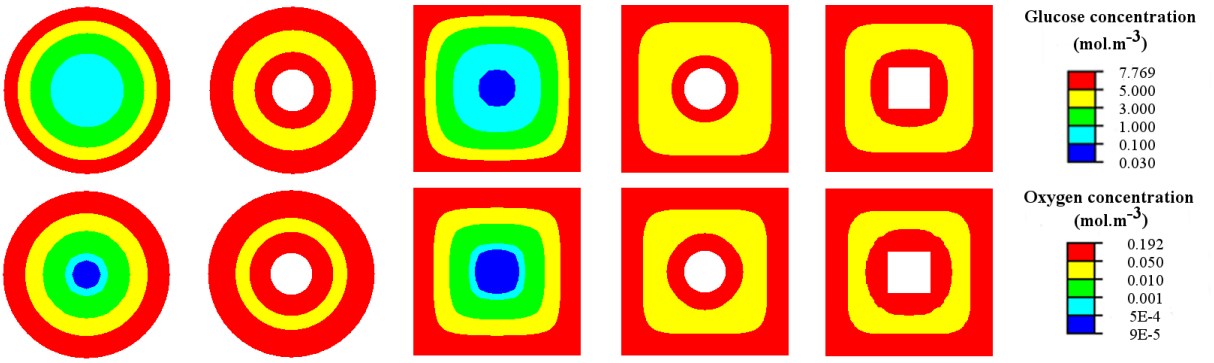

Figure 3: Glucose and oxygen steady-state radial distributions for the five different geometries. Profiles obtained at z=2.5 mm.

Regarding the solid constructs, the parallelepipedic shape has a more deficient diffusion of nutrients to the cores when compared with the cylinders. By adding a central channel, the minimum values of glucose concentration had a 46-fold increase in the cylindrical constructs and a 136-fold increase in the parallelepipedic constructs. Oxygen concentrations underwent a 200-fold increase of minimum oxygen concentrations in cylindrical constructs and 220-fold increase in parallelepipedic constructs. The increase in solute transport to the cores of the parallelepipedic constructs was slightly higher with a parallelepipedic central channel.

3.2 Cell density

The spatial patterns of cell density after 72h of simulation are shown in Figure 4. The spatial cell density profiles followed approximately the distribution of glucose with more populated peripheries and less populated cores. In the solid constructs, cell death in the cores dominated proliferation due to insufficient nutrient supply, while in the channelled constructs there was an increase of cell density relative to the initial seeding in all the points of the construct. The average cell densities increased at a higher rate in the channelled constructs. The highest proliferation values were achieved with the channelled cylinder (24% increase of cell density relative to the initial value). The introduction of channels led to an increase of 9% in average cell density with respect to the solid counterparts values after 72 hours.

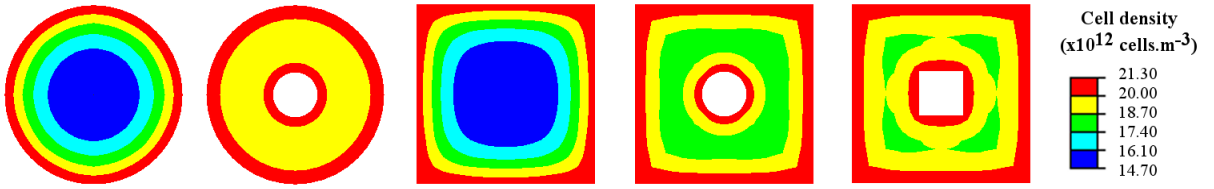

Figure 4: Cell density radial profiles after 72h of simulation for the different geometries. Profiles obtained at z=2.5 mm.

3.3 GAG & COL

The spatial profiles of GAG and COL had a similar distribution to the cell densities and limiting solutes, leading to peripheries with more ECM (data not shown). The production of GAGs and collagen is slow and, after 72h, the highest average values of GAG were about

0.06% w/w and the highest average COL concentrations were of 0.16% w/w. In agreement with the previously reported outputs, the introduction of a central channel has increased the global average concentrations of GAGs and collagen in the constructs. For both cylindrical and parallelepipedic scaffolds, the introduction of a central channel led to a relative increase of approximately 50% when compared to the solid constructs after 72 hours.

3.4 Biphasic mechanical properties

The spatial profiles of the Young's modulus (E) and the hydraulic permeability (k) are represented in Figure 5. The reported biosynthetic activity led to stiffer peripheries and softer cores. In solid cylinders, the mechanical parameters had negligible remodelling, while the peripheries had an increase of about 6.6 kPa (16.5% of the initial Young's modulus) and a decrease of 0.25 x 10^{-12} $m^4.N^{-1}.s^{-1}$ in permeability (5% of the initial permeability value). Overall, the introduction of channels led to a relative increase of 4% in the Young's modulus and of 2% in permeability after 72h in comparison with the solid constructs.

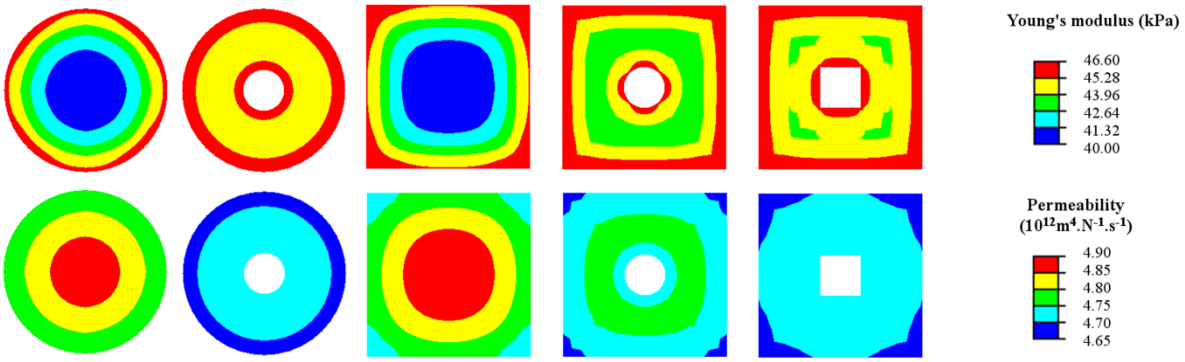

Figure 5: Radial distributions of the Young's modulus and permeability after 72h of culture for the different geometries. Profiles obtained at z=2.5 mm.

4 DISCUSSION

We have implemented a tool for sequential solution of the partial differential equations that define the biological activity of chondrocytes and mechanical properties' remodeling on tissue engineered cartilage constructs. This has proven useful to predict which scaffold geometry would promote a faster degree of tissue remodeling and the impact of the introduction of a single macrochannel on the biomechanical outputs.

Regarding the solid constructs, the values of the analysed outputs are higher in the cylindrical scaffolds and have a more regular distribution compared to the parallelepipedic scaffolds. One of the reasons for these differences is that, in the cylindrical scaffold, the difference from the centre to the periphery is 4 mm, while for the parallelepiped this distance oscillates between 4 and 5.66 mm, providing a longer and heterogeneous nutrient path length.

We have introduced a single macrochannel to increase nutrient diffusion to the centre of the construct. We chose channels of 2mm diameter/side since channels with diameters equal or superior to 1mm remain open for several weeks before becoming occluded with newly synthesized matrix, while they have a diameter lower that the critical defect size *in vivo*, i.e., defects with less than 3mm diameter are spontaneously repaired [4]. For this short simulation

time we could easily conclude that the introduction of a central macrochannel increased nutrient diffusion to the centre of the construct. The output distributions were more homogeneous and this positive outcome was particularly striking for the parallelepipedic scaffolds. Among the parallelepipedic scaffolds, the introduction of a parallelepipedic channel produced more homogeneous outputs and slightly higher global properties than the parallelepipedic case with the cylindrical channel. Again, these observations are due to shorter nutrient path length. However, the cylindrical constructs with cylindrical channels had the best biomechanical outputs of the group. The relative increase caused by the presence of the macrochannel was particularly noticeable in the GAG and COL global concentrations, with a 50% increase for all the channeled cases after 72 hours. However, the amount of ECM components synthesized after the end of the simulation period was below 0.07% w/w for GAGs and 0.16 %w/w for COL. These values are still very far from the native cartilage concentrations of 5-10 %w/w of GAGs and 10-20 %w/w of collagen [11]. Since the synthesized values of ECM components were very low after 72 hours of static culture for all the constructs, the remodelling of the mechanical properties was small (a maximum increase of 6.6 kPa for E and a maximum decrease of 0.3×10^{-12} $m^4.N^{-1}.s^{-1}$ for k). The estimated values for these biphasic mechanical properties are very far from the native cartilage properties [11]. The impact of the introduction of the channel in the global increase of Young's modulus and decrease of hydraulic permeability was very low, with variations of 4% in the channelled Young's modulus and 2% in the channelled permeability. It is plausible that, with longer culture periods, the differences in remodelling between the channelled and solid cases are going to be more notorious.

This article focused on the implementation of the spatiotemporal remodelling laws for static cultures. Mechanical stimulation has been shown to enhance the mechanical properties of TE cartilage when compared to static culture. With mechanical stimulation, fluid flow will increase the nutrient transport to the constructs, but it may also have a negative effect on the fixation of ECM components because, while increasing its synthesis, it may also increase its release to the culture medium [3]. This model does not reflect, in the synthesis of ECM components, the bound and free synthesized fractions, assuming only a total ECM fraction in the scaffold. A further expansion of the model accounting for these fractions when the dynamic stimulus is imposed will be derived.

Despite the fact that this work was developed from a theoretical standpoint, parametrization and validation for longer culture periods with experimental data is currently under way. However, we could qualitatively reproduce numerically the positive impact of reducing the diffusive nutrient path length on the remodelling of tissue engineered cartilage.

5 CONCLUSIONS

- We have successfully implemented a sequential finite element tool for resolution of the partial differential equations required for evaluation of the remodeling of tissue engineered cartilage in agarose-chondrocyte constructs and used it to evaluate the impact of the construct geometry and introduction of a macrochannel on the biomechanical outputs.
- Cylindrical constructs provide a more homogeneous diffusion path length than parallelepipedic constructs, therefore promoting slightly better global mechanical

properties.

- The introduction of a single macrochannel had a very positive impact on the homogenenization of the required outputs for cartilage remodeling, however the global relative impact after 72 hours of static culture on the mechanical properties is small.

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