MICRO-FINITE ELEMENT ANALYSIS OF HIERARCHICAL LAYERS OF THE ARTICULAR CARTILAGE FOR BIOFABRICATION

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Abstract: The biofabrication of engineered tissues is an essential area to reconstruct tissues. Until now, to the best of our knowledge, it has not been possible to mimic the biological and biochemical properties. New approaches to developing a new tissue become an attractive target for bioprinting, which is emerging as an essential strategy to recreate the histoarchitecture and the relationship between cells, matrix, and microenvironment. Simulations in a microscale study is a crucial factor to understand specifics properties. The objective is to analyze the impact of stress on the hierarchical layers of the cartilage using finite element method. The interaction of the collagen fibers with the chondrocyte was observed through the contact regions of the principal minimal stress analysis, mainly in the region of contact between chondrocytes. The deformation corresponds to the condition of constraint and applied force. The results are promising for future simulations of more detailed models with bias in vivo and in vitro.

Keywords: Micro-finite element method. Articular cartilage. Biofabrication. Computer simulations. Biomechanics.

1. INTRODUCTION

The biofabrication of engineered cartilage is an essential research area since these tissues are avascular, do not heal spontaneously, do not have a direct source of repair cells and is frequently subjected to harsh biomechanical stimuli. Because of these and by the low chondrocyte activity, when cartilage begins to degenerate or undergoes trauma, the lesions progress in a lengthy process, practically in an irreversible way (DUARTE CAMPOS et al., 2012). In this context, cartilage becomes an attractive target to bioprinting, which is emerging as an essential tissue engineering strategy to recreate the microphysical environment and the relationship between cells, their matrix and local anatomy (GAO; CUI, 2016).

Articular cartilage has an elaborate hierarchical structure that must be understood before the development of a mimicking structure can occur. Articular cartilage is hyaline cartilage and is 2 to 4 mm thick, and it is composed of a dense extracellular matrix (ECM) with a sparse distribution of highly specialized cells, the chondrocytes. The ECM is a complex network mainly composed of water, collagen, and proteoglycans, with other noncollagenous proteins and glycoproteins in lesser amounts (BUCKWALTER; MANKIN, 1998). Chondrocytes can assume different morphologies and collagen fibers can be distributed in different orientations. Then, was identified in whole cartilage tissue, various levels in the hierarchical structures, called zones, with synergic interactions between these different levels, shown in Fig. (1) (MARDONES; JOFRE; MINGUELL, 2015).

The mechanical properties of cells and the collagens are essential in the regulation of many aspects of the ECM (LU; MOW; GUO, 2009; SILVER, 2006; WU; HERZOG; EPSTEIN, 1999). Besides that, the functions of articular cartilage compromise the rheological viscoelastic properties of cartilage when it is subjected to constant stress. Therefore, there is a great need for research into the mechanical behavior of biological materials in order to attempt to correlate the development of biological tissues, such as cartilage, and the proclivity and progression of specific diseases to the mechanical behavior.

Until now, to the best of our knowledge, it has not been possible to mimic the biological and biochemical properties of articular cartilage. In this context, computational simulations can be performed to propose a model for three-dimensional microarchitecture to understand this tissue. In the sequence, with a layer-by-layer assembly, the three-dimensional tissues with complex structures can be printed using biological images and multiscale simulations to study the biomechanical profile (DERNOWSEK et al.,2016; ANDRÉA DERNOWSEK; REZENDE; LOPES DA SILVA, 2017; DERNOWSEK; REZENDE; SILVA, 2017). The use of modeling and simulations in microscale is a crucial factor to understand specifics properties of biological tissue. Then, the objective of this study is to analyze the impact of stress on the hierarchical layers (in a microscale) of the articular cartilage using micro-finite element (MFE) simulations.
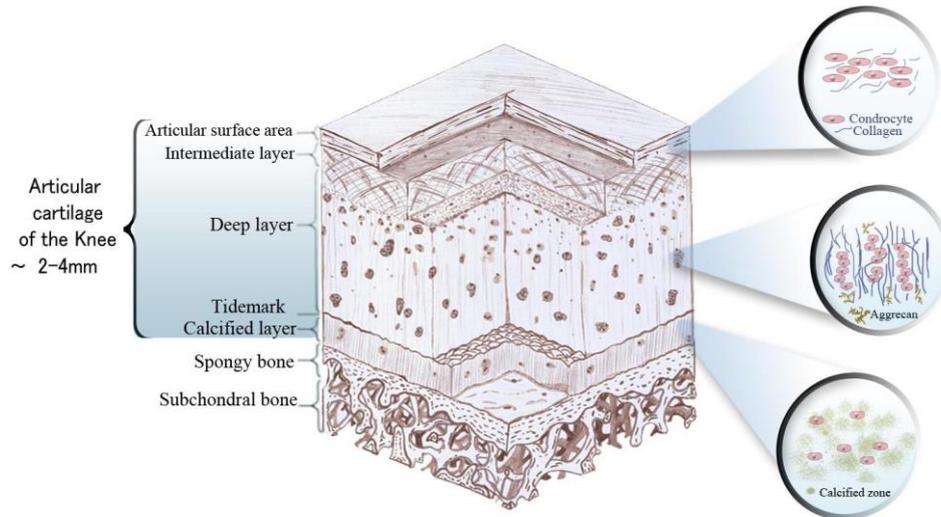
Figure 1. Schematic, cross-sectional diagram of healthy articular cartilage: cellular organization in the zones of articular cartilage and architecture proteins.

2. METHODS

We developed an anisotropic and microstructural finite element model of some components of articular cartilage to applied pressure. The variation of the physical behavior of the model, an anisotropic characteristic, will be verified from the geometric configuration of the collagen fibers among the chondrocytes, in order to mimic the real microstructure of interstitial materials with isotropic properties (Tab.(1)).

The microstructures of the articular cartilage layers modeled in the Rhinoceros® 5.0 (McNeel North America, Seattle, WA, USA) software, and the file in format .stp (step) was imported into Ansys 17.2 (ANSYS Inc, Houston, TX, USA) for the finite element analysis (FEA).

The model (BioCAD) consisted of the extracellular matrix – type II collagen - and chondrocytes located in the deep zone. This zone was chosen because corresponds to a 45% of the ECM volume. Chondrocytes were surrounded by a collagen matrix, a hydrogel and were assumed spherical before load application. Material properties of the chondrocyte and the ECM were obtained from the literature. The materials were considered isotropic, the BioCAD of cartilage components and the boundary conditions are summarized in Fig. (2). Contact regions between the cartilage components – cells, collagen, and interstitial fluid (~ water) – they were considered correctly bonded.

The Fig. (2a) shows the important aspects of the hierarchical layer of the deep zone of articular cartilage, such as the orientation of collagen fibers arranged perpendicularly to the articular surface, chondrocytes in the spheroidal shape and arranged in radial columns aligned with the collagen fibers. The biological properties and dimensions used of cartilage components are listed in Tab. (1) and (2).

Table 1. Mechanical properties of articular cartilage components.

Components	Young's modulus (MPa)	Poisson coefficient	References
Chondrocyte	0.001	0.2	(WU; HERZOG; EPSTEIN, 1999)
Type II Collagen	1520	0.1	(SILVER, 2006)
Proteoglycan	0.070	0.08	(LU; MOW; GUO, 2009)

Table 2. The dimensions used for finite element analysis

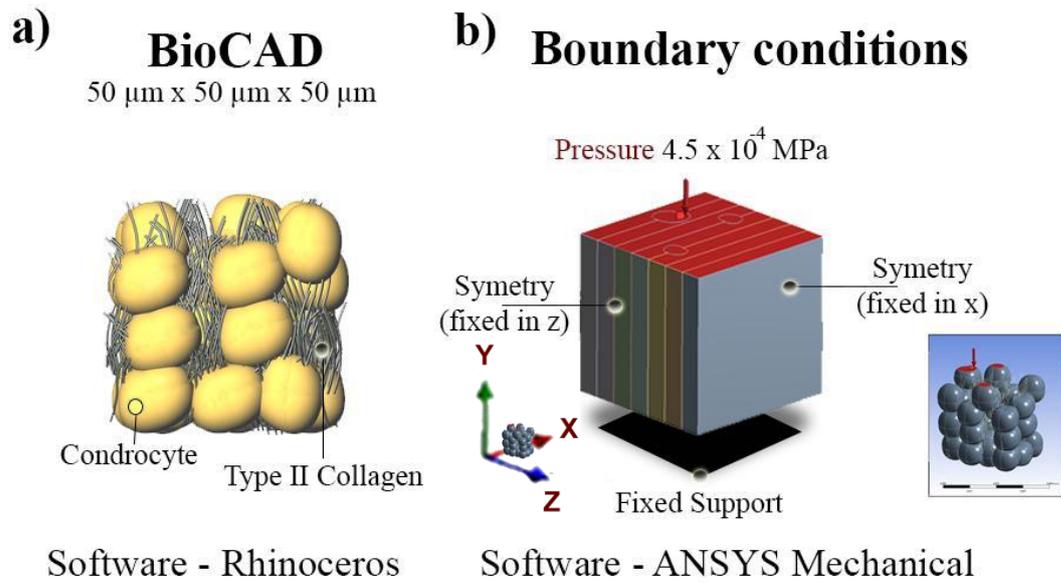| Components	Dimension (μm)
Chondrocyte diameter	10 (LI, 2014)
Type II Collagen length	1
Type II Collagen diameter	0.4
Cartilage homogeneous unit	50 x 50 x 50

Figure 2. Micro-finite element analysis of cartilage components regarding CAD Modeling (BioCAD) made in Rhinoceros software (a) and Boundary conditions modeled in ANSYS software (b).

3. RESULTS AND DISCUSSION

Cartilage's component showed a promising technique *in silico*, using the MFE analysis. This model included the chondrocytes and collagens of the deep layer of the articular cartilage and material properties of these structures, to predict the internal mechanical response of the tissue in a multiscale study. Cell and collagen deformation metrics can be extracted from simulation results to provide a simplified description of individual components responses. The 3D computational domains were initially discretized into 480,081 elements Fig (3).
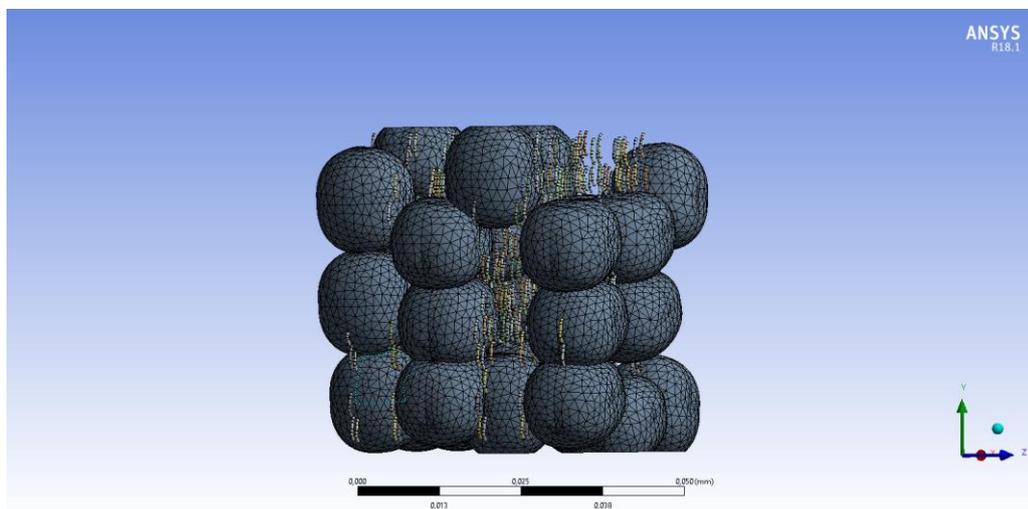

Figure 3. Computational meshes used for studies

Skewness is defined as the difference between the shape of the finite element and the shape of an equilateral finite element of equivalent volume. Highly skewed finite elements can decrease accuracy and destabilize the solution. A general rule is that the maximum skewness for a tetrahedral mesh in most flows should be kept below 0.95, with an average value that is significantly lower. The mesh quality of the model obtained, using the skewness command of Ansys is demonstrated in Tab. (3):

Table 3. Skewness value

Skewness Value	Number of Elements (%)
0.05	53900 (11.22)
0.15	128978 (26.86)
0.25	122863 (25.60)
0.35	88300 (18.40)
0.45	47100 (9.81)
0.55	21100 (4.40)
0.65 to 0.95	17840(3.71)

The collagen fiber contribution can be observed by analyzing the deformation and minimum principal stress (Fig. 4 and Fig. 5). The results suggest that elongation of the collagen fiber - the major cartilage´s component - may involve a change in the ECM structure, probably because the collagen fibers interact with several molecules.

The deformation analysis in Fig. 4, allows us to visualize which regions are moving in response to the applied pressure. It was verify that there was a gradient of displacement along the whole structure. The minimum main tension in Fig 5 is to emphasize the areas that undergo significant compression efforts, allowing to evaluate how the chondrocytes and the collagen distribute the efforts in the system. So, there is an increase in the compression stress around the chondrocyte contact region (highlighted in green) with the value of -6.3208*10-4MPa.
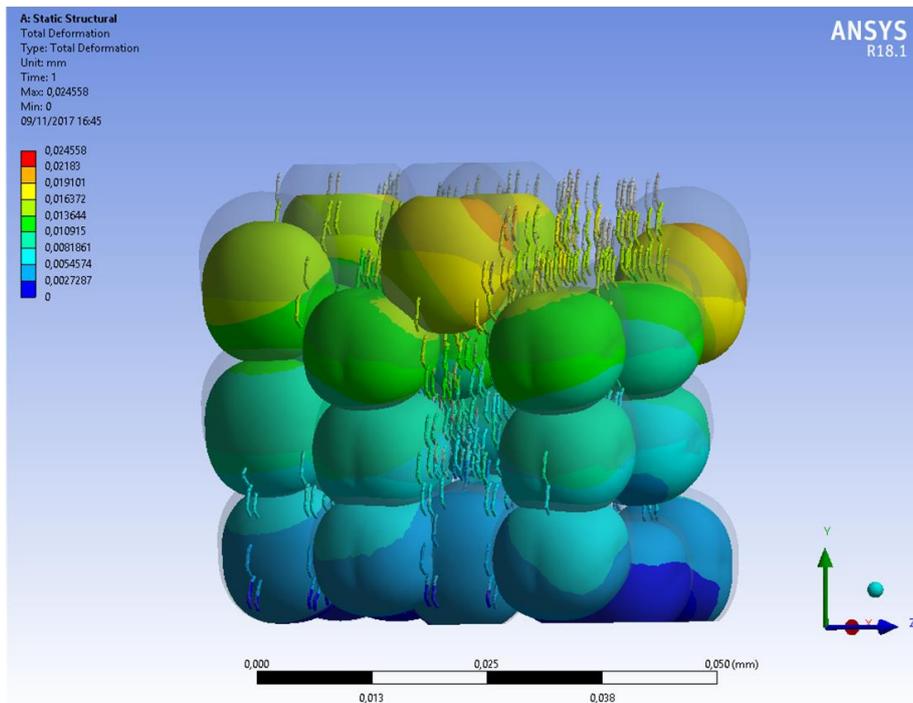

Figure 4. Results plots are showing microscale deformation of cartilage`s components in the deep layer.

The collagen network and proteoglycan matrix are thought to play an essential role in controlling the stresses and strains in and around chondrocytes, in regulating the biosynthesis of the ECM, and consequently in maintaining the health of biomechanical properties (KORHONEN et al., 2008). Understanding the detailed effects of the mechanical environment of chondrocytes on cell behavior is therefore essential for the study of the development, adaptation, and degeneration of articular cartilage.
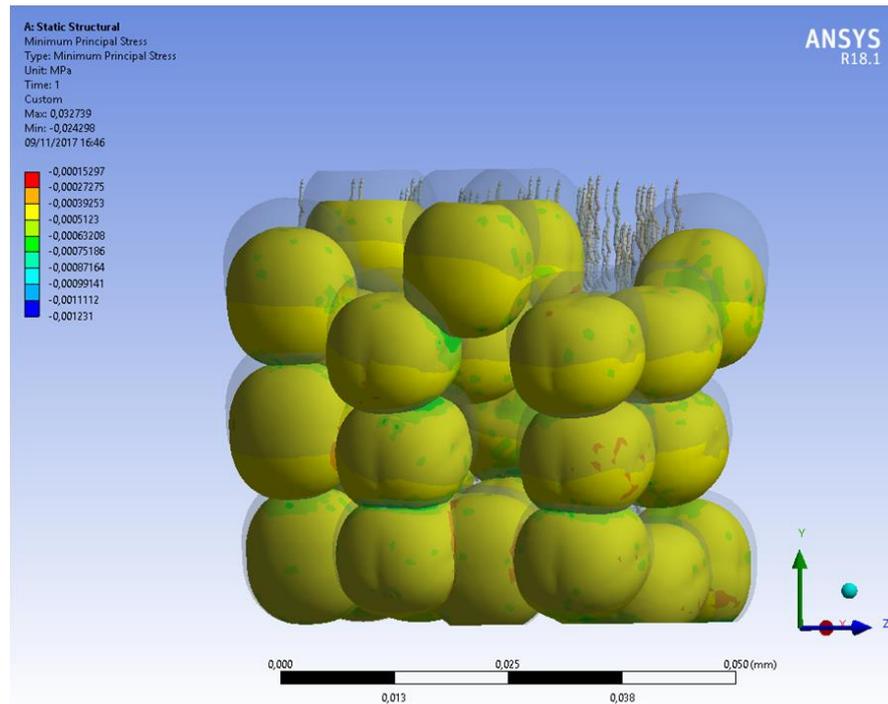
Figure 5. Results plots are showing minimum principal stress of cartilage`s components in the deep layer.

The *in silico* results of the deformation profile of chondrocytes and collagen fibers against external pressure will provide information indicative of the mechanical response of the modeled tissue, which will serve as a guide for the construction of cartilaginous tissue structures *in vitro* and *in vivo*.

4. CONCLUSIONS

Although finite element simulation and analysis confirmed fibril assembly, the application of this technique in our study for tissue modeling and simulation can be considered in an incipient stage. Computational modeling and simulation provide descriptive and predictive tools to identify multiscale interactions, and can lead towards a greater comprehension of healthy and diseased cartilage function, possibly in an individualized manner. The exploration of micromechanics of cells and biological structures helps us to understand a range of processes such as disease progression and cell–materials interactions for biofabrication of tissues and organs. Knowledge of the effect of the biomechanical environment of chondrocytes and the ECM, combined with tissue engineering and clinical research, should directly lead to better techniques for cartilage repair and improved strategies for cartilage matrix production. Finally, these studies provide essential information for cell biology, tissue engineering, biofabrication, and bioprinting areas.

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5. ACKNOWLEDGMENT

6. RESPONSIBILITY FOR INFORMATION

The authors are solely responsible for the information included in this work.