# A framework for multiscale bioengineering simulation

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**Abstract**

*Research Article*

*Computer Simulation for biological and bioengineering purposes is being referred today also as In silico as a first approach for the In Vitro and In Vivo expensive and time-consuming tests. It is a necessary and fast-growing field of interdisciplinary knowledge to fulfill today's and future demands, ranging from bioengineering modeling and simulation of mechanical devices to the more complex and uncertain field of biofabrication of human tissues and organs towards we call Medicine 4.0. In order to establish models for the phenomena studied, not only the boundary conditions and mathematical representation of such chemical, biological, biochemical and physical phenomena has to be implemented, but the computational approach to obtain representative models and relevant results as a feasible task. Such models can bring solutions to one level of complexity according to the specific problem to be solved. On the other hand, it is practically impossible to establish more complex models for high-level solutions as the ones necessary for complete organisms and organs simulations, for example, due to its complex integration in many levels from molecular to the high-level behavior of such organisms and organs. Therefore, like engineering approach, it is mandatory for future solutions the integration of multiscale models from molecular to whole organism levels. This paper sheds some light on this subject not only proposing a preliminary framework as a basis for multiscale simulation but also showing, using case studies, the complexity and necessary simplification for some of those levels involved in a possible framework from bioengineering to biological multiscale simulations. The case studies presented in this paper are only a small set of models illustrating the challenges and needs for a complete integrated framework. They were developed initially as a specific demand in a stand-alone approach at 3D Technologies Research Group in the Renato Archer Information Technology Center.* **DOI**: *https://doi.org/10.24243/JMEB/2.5.177*

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# 1 Introduction

Multiscale simulation is one of the emerging and challenging areas in the bioengineering studies for complex systems modeling. Considering the modeling of such systems a highly complex taskis essential to start with therelativelyless complicated approach and definitions taking into account only one scale level of a system or a subsystem and the corresponding integration among levels. There are many steps necessary to study in silicobioengineering systems and, in such cases, the use of mechanobiology.

Knowledge, biomechanical data, computer-aided design (CAD) and computational models are required. The biological phenomena present in bioengineering problems are a combination of multiscale interrelated components such as molecules, regulatory networks, cells, tissues, whole organism, biomechanical properties, among others [1].

Fundamentally, a multiscale simulation must explicitly explain more than one level of complexity in measurable domains [2]. Under those circumstances, material properties are common issues when dealing with computational simulations based on mathematical models which, in general, utilizes equations that intends to represent the reality-based on standard characteristics, obtained by patterned procedures which make possible to reproduce the results. One of the primary constraints of this assumption is whether or not the real material considered can be represented by its standardized properties like elastic modulus, Poisson ratio or any other necessary to perform simulations. For simulations in conventional mechanical engineering applications, some standards provide such warranties, as well as control systems that keep variability under acceptable values related to quality and interchangeability [3].

The standardization is a reasonably easy task to achieve when one designs the application and the solutions together with well-known materials, which are the case in conventional engineering applications. On the other hand, in the bioengineering studies, an intrinsic variability of nonconventional materials must be handled due to the dynamics of biological components present in living beings. Therefore the conventional approaches, using standard engineering procedures, do not suffice when used to obtaining mechanical properties from an organic material. Usually, the result is a variety of complex values which presents no immediate convergence, reflecting the actual variability of the biological entities on which the achievement of the organic material properties is basedin. Currently, the modeling of this kind of complexity has been made by trying to fit them into consolidated mathematical models, making possible to define a set of mechanical material properties that can be achieved by standardized mechanical essays. This procedure can be found in many bioengineering references related to mechanical characterization of biological materials [4]-[8].

In this context, organic materials are components of biological entities – genes, proteins, cells, and tissues - resulting from many processes and biochemical interactions guided by multi-omics mechanisms. Through these ideas, such microscale additive manufacturing has high controlled cycles, which include synthesis of microscale material by selective deposition of multiple components, producing complexity based on the combination of simple materials. Such microscopic level building responds to localized conditions and stimuli, resulting in complex macroscopic behavior, optimized by continuous biodynamic processes.

One of the challenges in the current era of data abundance is to understand how the interactions between discrete biological system components result in integrated emergent effects on higher order systems changing the biomechanical properties. This paper presents some specific and stand-alone computer simulations approaches and applications as well as a discussion focused on in silico  multiscale approaches of bioengineering systems, which are developed at 3D Technologies Research Group (NT3D), to predict a myriad of biological and biomechanical phenomena, besides to provide a new outlook in the bioengineering field, considering the future integration of such applications in a framework.Figure 1 shows the multiscale approaches that have developed at NT3D.
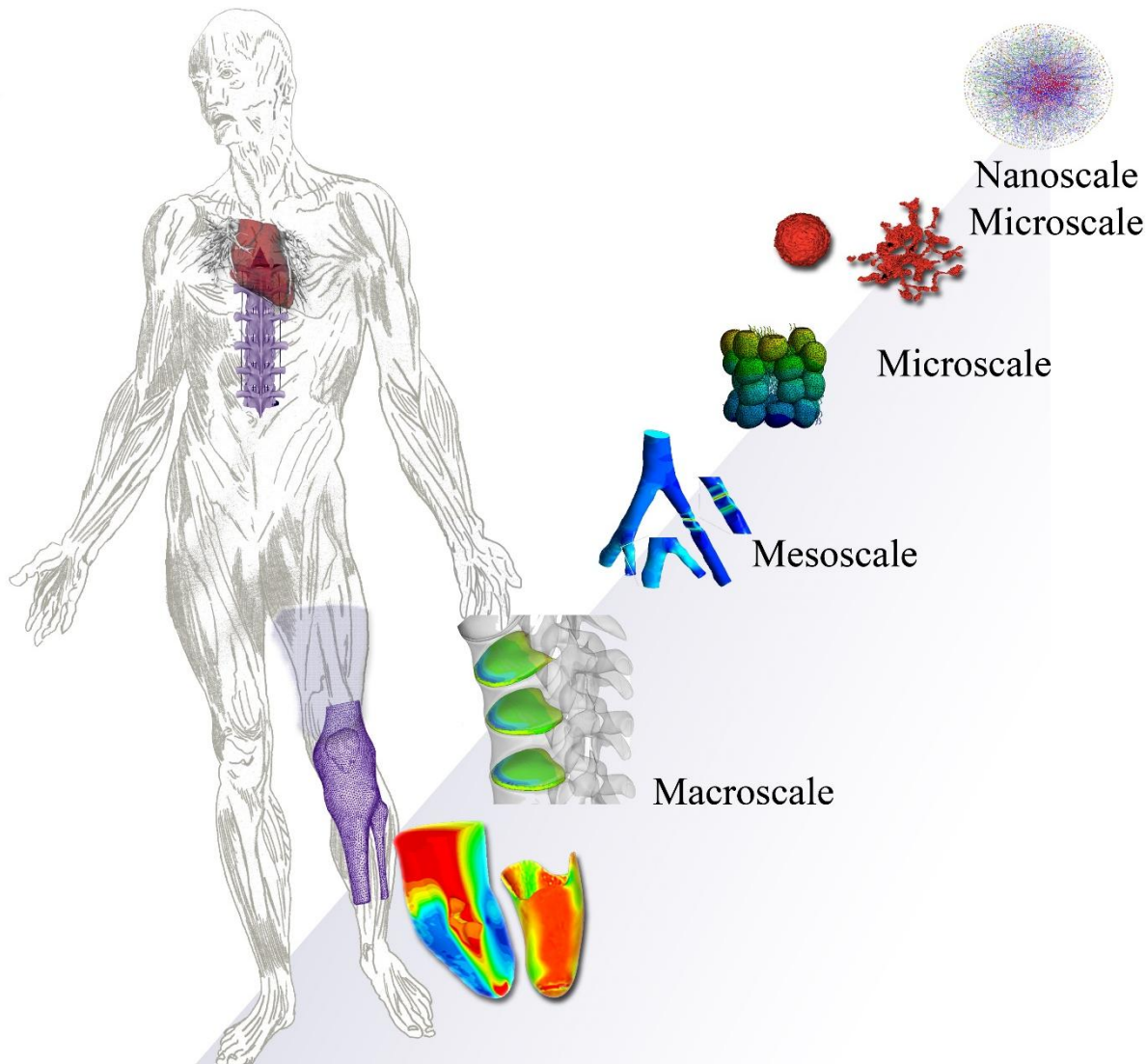
**Fig. 1 Multiscale approaches developed at 3D Technologies Research Group (NT3D)**

### 1.1 Nano to MicroScale Bioengineering Simulations

#### 1.1.1.    Study 1 - Biomarkers Analysis and Selection Using Open Platforms

In recent years, nano and microscale studies have been carried out in some fields offering new opportunities for discovering and investigation, helping and improving the storage, organization, and classification of enormous data sets of digital information available on the Internet. The complex system, as a characteristic of the biological tissues, needs to be investigated carefully, to properly orchestrate the diverse processes such as multi-omics networks, cells, cell aggregation, proliferation, differentiation, angiogenesis, mechanobiological properties, among others [1].

There are many *in silico* approaches like data mining and machine learning to understand and predict organic materials characteristics in nano and microscale, identifying new specific biomarkers of a biological process. Consequently, these tools use a set of statistics, machine learning and mathematics models to identify patterns and trends, which can not be easily discovered with traditional methods. Such methods provide an *in silico* counterpart building predictive models based on chemical structure features and other properties, and training sets of known biomarkers [9].

Although data mining techniques were used in various studies in genetics field, their application for biomechanical purposes remains few explored. Nano and microscale *in silico* approaches can be studied applying data mining techniques in a knowledge discovery process to identify specific biomarkers of a biological process. In our approach, as shown in Figure 2, the following steps were executed: (1) data source selection, (2) preprocessing, (3) transformation, (4) data mining, and (5) the interpretation/evaluation of the results.

In the first step, we selected omics data from The European Bioinformatics Institute databases (EBML-EBI), which encompasses data from many life science experiments. Gene expression underlying Osteo-, Adipo-, and chondrogeniclineage commitment of humanmesenchymal stem cellswere selected for analysis.The microarray data of this study are available at ArrayExpress Data Bank (https://www.ebi.ac.uk/arrayexpress) under Array Express accessions E-MTAB-3731. It contains Osteo-, Adipo-, and chondrogenic differentiation data and expression measurements in triplicate time. The total of 12.083 genes including cell control measures within minutes to hours periods was analyzed during the preprocessing step. We filtered and standardized the data source to avoid divergences and to provide a standard data structure necessary for the next steps. For instance, the median of the hourly measures was considered to reduce the final amount of attributes. Thus, the data was organized by hourly intervals to standardize each analysis data. In addition, each gene was labeled according to their identification on National Center for Biotechnology Information (NCBI).

In sequence, a literature review was conducted aiming at to find genes already known as expressed for osteogenesis. This survey found only 151 genes. This gene data set is necessary for training machine-learning algorithms and for testing their performance. We selected only the classifiers that obtained accuracy higher than 70% (using the testing set) to apply to the entire database. The classifiers selected are Logistic, Multilayer Perceptron, Multiclass Classifier, OneR, and RandomForest. Then, the genes were ranked according to the median of the prediction values of these classifiers. Positive prediction values were adopted for expressed genes and negative predictive values for non-expressed ones (e.g., a gene with +0.80 means that a classifier considered that it has 80% of probability to be expressed). Finally, we evaluated the first 20 genes.
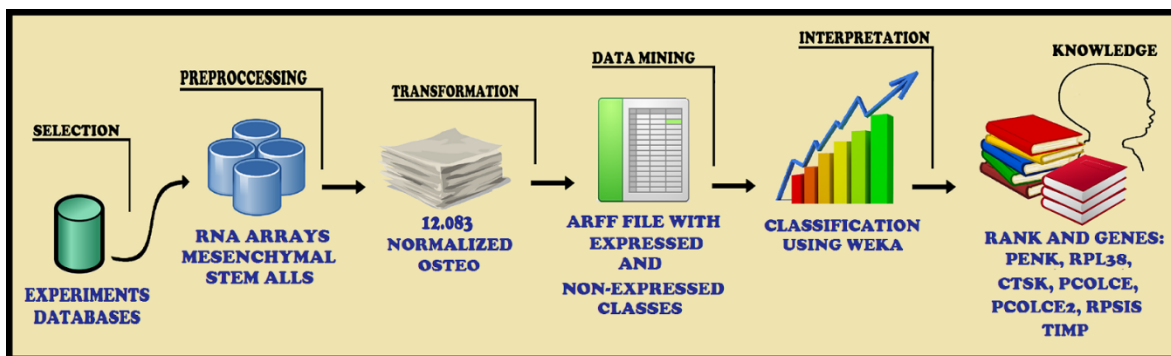


*Fig.2Workflow of the methodology for Discovery Process. The steps are (1) experimental databases (2) preprocessing, (3) transformation, (4) data mining, and (5) the interpretation/evaluation of the results.*

Our study obtained a complete ranking of the 12.083 genes analyzed, which 104 genes had more than 0.99 of median prediction values obtained in the selected classifiers. We found values related to the bone expression for seven genes from the 20 best classified, as follow: PENK, RPL38, CTSK, PCOLCE, PCOLCE2, RPS15, and TIMP.The results of the classification were promising for the analyzed set, indicating the possibility of applying this approach for other types of biological studies. The main limitation concerning the use of the data mining algorithms were the size of the training set when new data about the expressed genes are available, possibly the accuracy of the algorithms will increase too, as well as better prediction rates will be obtained.
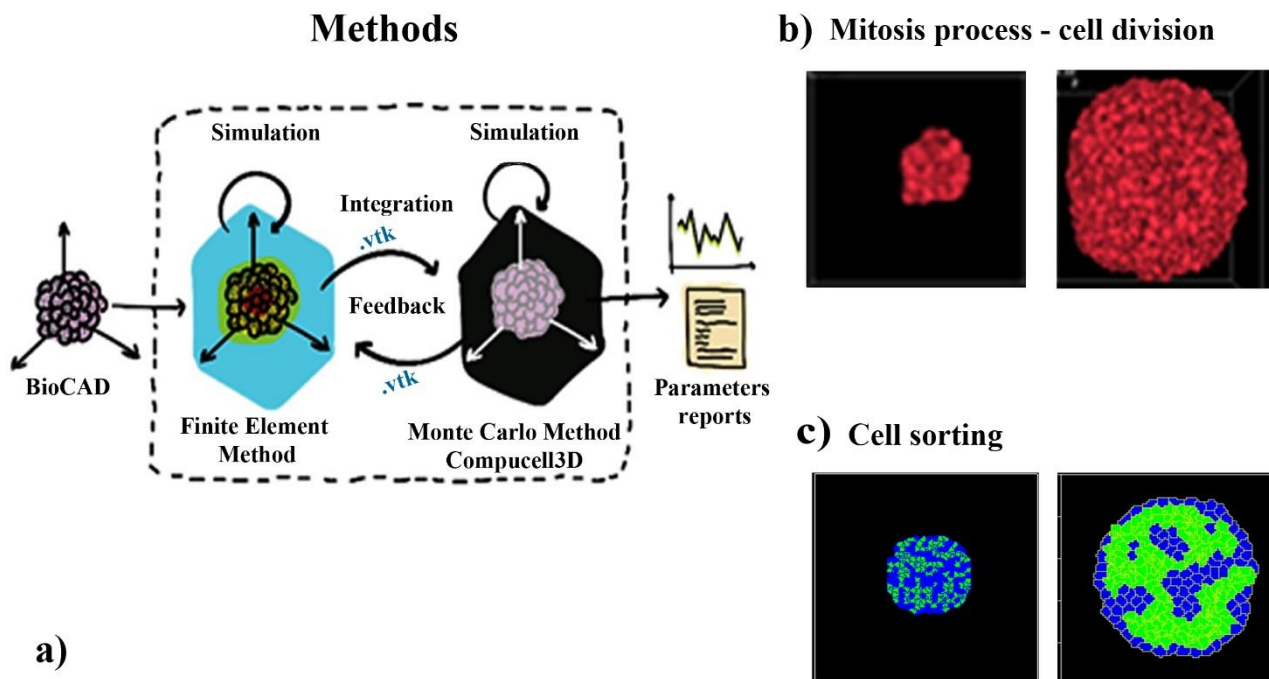
Therefore, the use of the multi-omics data, data mining and machine learning as *in silico* tools, allow predicting structure-function of biomarkers and the possible biomechanical characteristic.

### 1.1.2. *Study 2 - Tissue Spheroid Simulation for Angiogenesis*

Tissue engineering and the bioengineering assist in the modeling of biological systems, initially studying the behavior of a basic living unit like tissue spheroids and then moving into its relationship with others neighbors spheroids to form a complex tissue. A range of multiscale strategies was employed to develop a BioCAE for bioengineering studies preventing a significant amount of trial-and-error experiments in laboratories [1].

Tissue spheroids are cell clusters used to study a myriad of biological behaviors, mainly in the cancer biology. Compucell3D (CC3D) [10] is a software for biological simulations using mathematical models to represent living cells and mimic some behavior observed in biological organisms. The CC3D is used to model and simulate micro and mesoscale biological structures, like tissue spheroids by changing parameters such as cell number, cell volume, contact energy between them and total spheroid radius (figure 3a). The study presented in this paper has focused on the angiogenesis of endothelial cells aggregate (microscale study) to understand the chemotaxis of endothelial cells inside a 3D environment. Cellular Potts Model was used to model cell behavior and the agent-based models for interaction among entities. This biological study uses complex phenomena of cellular interaction to analyze behaviors such as cell division, diffusion, and chemotaxis of the basic units that make up the tissue. For this, the algorithm makes a copy of each pixel and overlaps by a randomly chosen neighbor. If the new grid configuration reduces the system's total energy, the change is maintained.



*Fig.3 Nano to microscale simulations using biological data and mathematic methods; a) Scheme of the methodology applied in this case; b) Simulation of the tissue spheroid evolution – mitosis process; c) Simulation of cell sorting of a tissue spheroid.*

The first graph, generated by CC3D scripts, shows the expected exponential growth of the number of cells in the spheroid due to mitotic behavior (Figure 3b). The number of cells is very relevant to decide, for example, for some nutrients that should feed that cell cluster in a given context. Figure 3c shows us another example of biological phenomena that can be studied using this methodology. Cell sorting is a biological process that allows the polarization of cells based on different physico-chemical properties.

For *in silico* angiogenesis assay, spheroids were generated using CC3D, to analysis cells extended filopodia into the virtual environment stimulated with Vascular Endothelial Growth Factor (VEGF). Proangiogenic growth factors such as VEGF stimulate quiescent endothelial cells to form tip cells, which form filopodia, proteolytically degrade their surrounding extracellular matrix, and migrate toward the angiogenic stimulus. The results of the simulation were compared with *in vitro* angiogenesis experiment developed by Heiss and collaborators in 2015 [11]. The comparison of *in silico* results and *in vitro* experiment demonstrated the feasibility of simulation to replace some experiments with definite similarity like sprouting and elongation of cells (Figure 4).
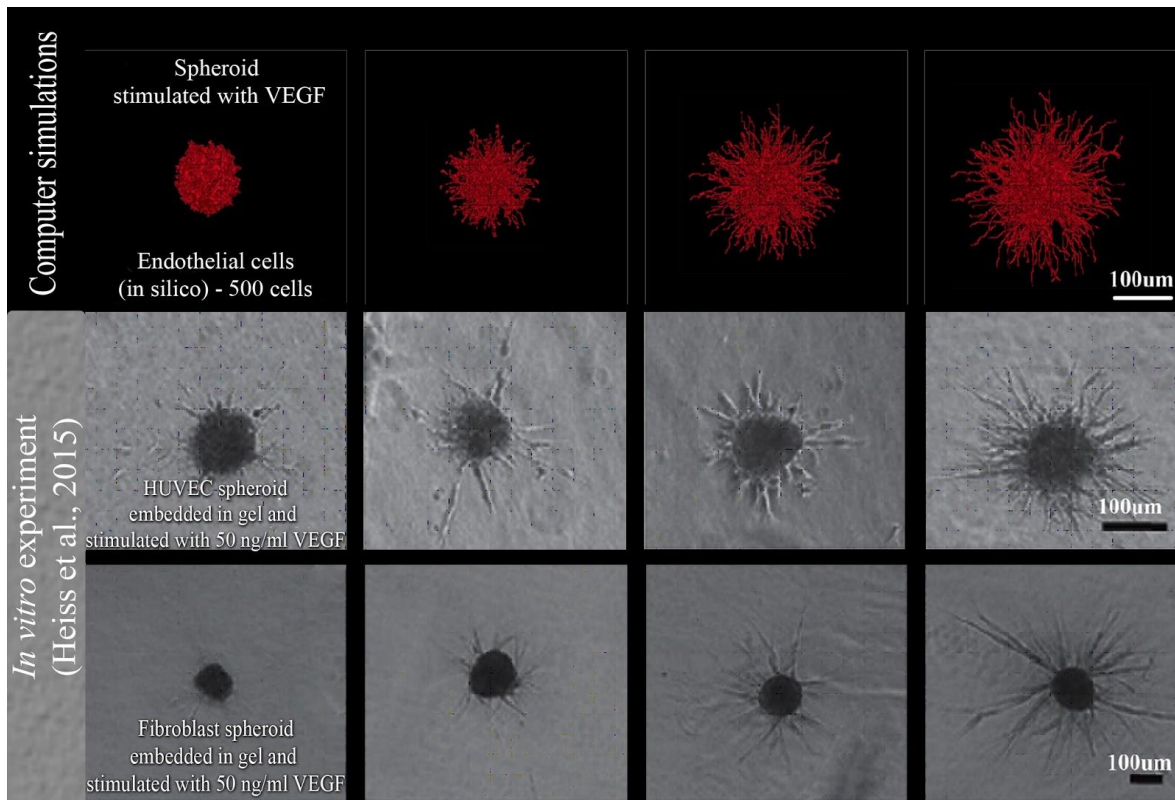


*Fig. 4 Comparison between in silico and in vitro angiogenesis. Both the in vitro (10)and in silico experiments used the VEGF to stimulate the cells.*

The integration of mechanical and biological software brings to the *in silico* multiscale approach a variety of parameters that aid the decision to apply bioengineering methods, since they are still very new and needs continuous improvement until we can reach a complete tissue simulation in the future [12]. The emergence of integrated platforms on different systems levels to understand complex processes will enable the prediction and creation of better biomodels.

### 1.1.3.   Study 3 - Cartilage Mechanical Simulation

The tissue engineering of cartilage field is an essential research area since these tissues are avascular, do not heal spontaneously and do not have a direct source of repair cells. Because of this 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 [13]. In this context, the cartilage becomes an attractive target for bioprinting approach, which is emerging as an essential tissue engineering strategy to recreate the microphysical environment and the relationship between cells, their extracellular matrix (ECM) and local anatomy [14]. The mechanical properties of cells and the collagens are essential in the regulation of many aspects of the ECM [15]-[17]. Thus, the use of modeling and simulations in a microscale is a crucial factor to understand specifics properties of a biological tissue. The objective of

this study is to analyze the impact of stress on the hierarchical layers (microscale) of the articular cartilage using micro-finite element (MFE) simulations.

The microstructures of the articular cartilage layers were modeled in the Rhinoceros® 5.0 (McNeel North America, Seattle, WA, USA) software, and the '.step' the file was imported into Ansys 17.2 (ANSYS Inc, Houston, TX, USA) for the finite element analysis (FEA).The boundary conditions are summarized in figure 5 and the contact regions between the cartilage components – cells and collagen –they were considered correctly bonded.Finally, the mesh hasa total of 480,081 elements.

The biological properties of cartilage components are listed in *Tables 1 and 2*.

**Table 1. Mechanical properties of articular cartilage components**.

| Components | Young's modulus (MPa) | Poisson coefficient | References |
|---|---|---|---|
| Chondrocyte | 0.001 | 0.2 | [15] |
| Type II Collagen | 1520 | 0.1 | [16] |
| Proteoglycan | 0.070 | 0.08 | [17] |

**Table 2. The dimensions used for finite element analysis**

| Components | Dimension (µm) |
|---|---|
| Chondrocyte radius | 10 |
| Type II Collagen length | 1 |
| Type II Collagen diameter | 0.4 |

Cartilage's component can be studied*in silico*, using the MFEanalysis.Cell and collagen deformation metrics can be extracted from simulation results to provide a simplified description of individual components responses. 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 multiscalar study.

We can observe the collagen fiber contribution by analyzing the deformation and shear stress behavior (Figure 5). The results suggest that collagen fiber stretching may involve a change in the structure of ECM, probably because collagen fibers interact with several molecules.

## 2 Mesoscale

### 2.1 Study 4 - Computational Fluid Dynamics of a vascular branch

Computational fluid dynamics (CFD) is one of the most well-known computational methods to solve continuum fluid flow simulation. In biomechanics, its utilization for research in blood flow analysis is already in use. The critical issue to model blood flow in mesoscale or microscale is the impossibility to consider all blood constituents [18], [19]. In order to model the blood flow simulation in mesoscale, selection of suitable blood constituents need to be found.

Blood flow in vascular segments is of great interest to biomedical researchers because the biomechanical force profile generated by flow is thought to play a significant role in vascular remodeling and graft failure. Vascular branches with bifurcations and thickening are significant regions to understanding the blood flow patterns and deal with vascular problems is one of the most challenging tasks in vascular medicine [20], [21]. The vascular diseases result from an abnormal narrowing of blood vessels, and it brings the most effective changes in pressure, velocity, wall shear stress (WSS), and impedance on the blood flow, influenced by the different geometries [22]. Thus, the purpose of this study was to explore the effect of hemodynamic factors in typical vascular segments and to compare the WSS profiles with different models of vascular stenosis using mesoscale simulations.
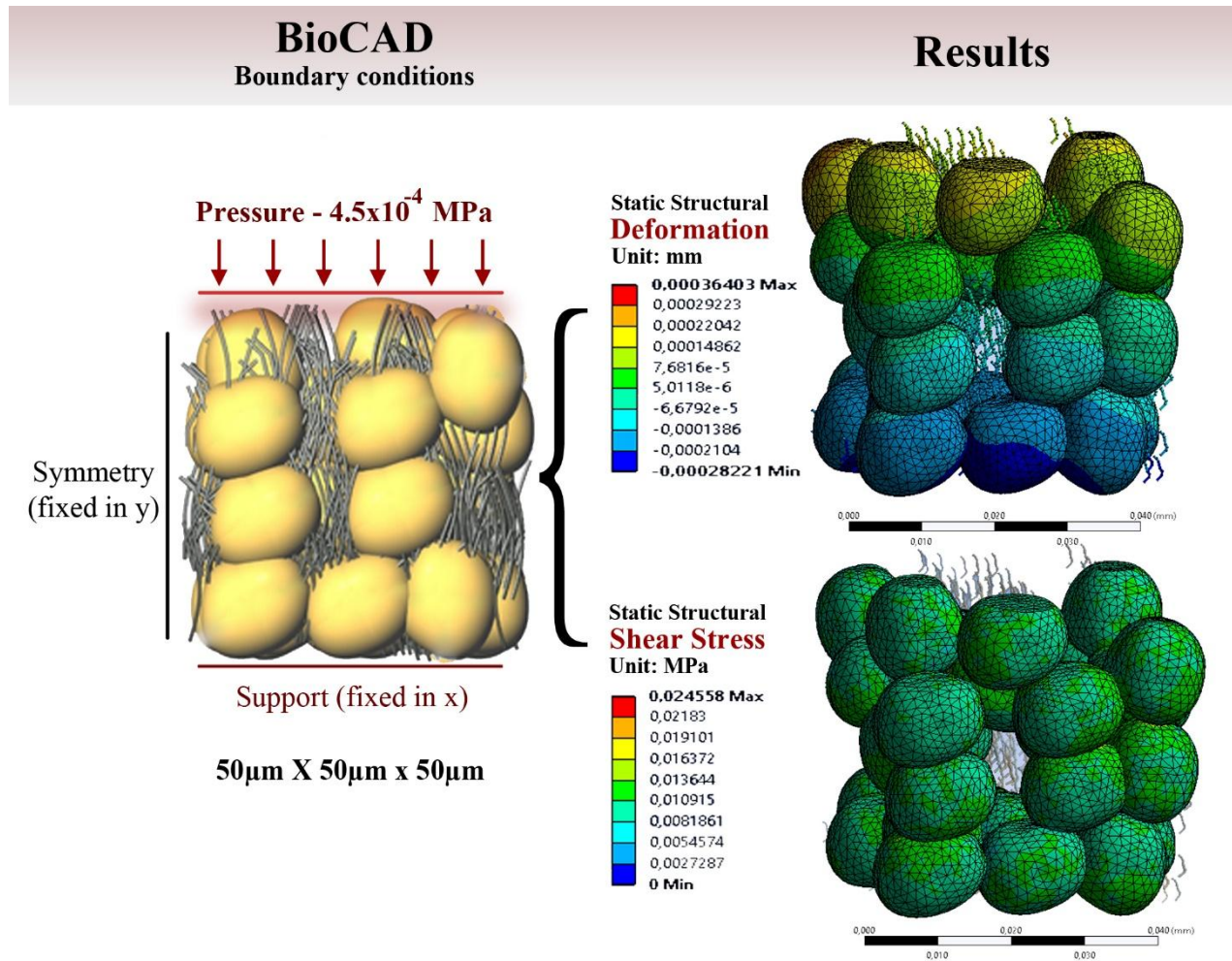
***Fig. 5 Micro-finite element analysis of cartilage components regarding CAD Modeling (BioCAD) and results - deformation and shear stress analysis.***

The vascular model used in our study started by reconstructing a vascular branch located near to the heart. This ramification came from a Computed Tomography (CT) scan. The medical imaging open-source software called InVesalius, created by our group [23] can import CT images and generate a 3D digital model of a specific anatomy. Two stenosis geometries were modeled as a centered change in the artery diameter. In order to generate a mesh for simulation, the surfaces were modeled in the Rhinoceros® 5.0. Numerical algorithms structure CFD codes are governed by the Navier–Stokes equations. The studies showed the impact of the blood flow in different models of the vascular branch.

The models used in this study and the dimensions of the vascular branches are illustrated in the figures 6a. The rheological property of blood flow was assumed to be laminar, viscous, incompressible and Newtonian, with a density of 1050 kg/m$^3$, viscosity of 0.0035 Kg/m.s$^{-1}$ and velocity (inlet) of 333 mm/s. The wall is rigid satisfying no-slip conditions. The outlet boundary condition is the opening where the average pressure is assumed as zero. The real mean pressure cannot be zero. However, this zero assumption is widely employed in CFD simulations, as it should not influence the flow results. The residual convergence is designated as 10$^{-6}$. The hemodynamics simulations are performed using ANSYS-CFX 17.0,and the 3D computational domains were initially discretized into elements in the range of 219,000 and 450,000

Previous studies have suggested that the hemodynamic wall shear stress profiles influence the magnitude and pattern of stenosis regions [24]-[26]. For this reason, the described study has focused on the impact of the blood flow in the wall vascular of a healthy subject (normal) and the arteries stenosis (regular and irregular).

In our studies, the WSS magnitude was predicted (figures 6b-d) and exhibited a visible increase in the area of the stenosis. In the irregular stenosis, an increase of the WSS was found (figure 6d).These results suggest that the differences in WSS between normal and stenosis modelsare visible, and corroborate with many previous studies. All in all, these data provide valuable preliminary information for future hemodynamic studies regarding thestenosis regions.
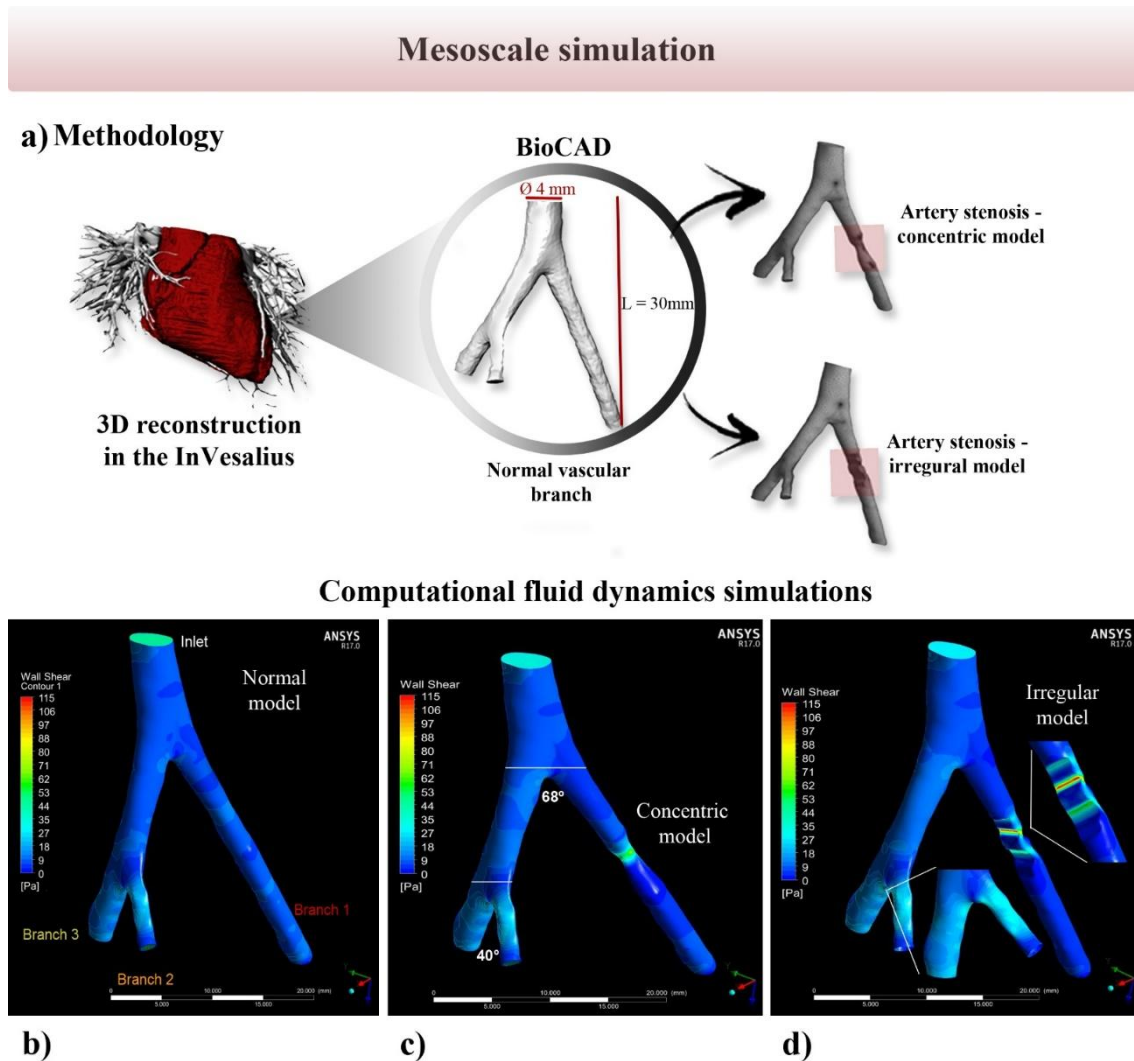


*Fig. 6.a)Three models were used in this study: the normal model and two vascular stenoses (concentric and irregular models). Wall shear stress (WSS) profile of three models; In the typical vascular branches model; b) a low WSS was observed along the sidewalls. In the concentric and irregular stenosis models; c-d) the wall shear contour show a significant increase in the regions modeled.*

In general, studies like these demonstrate that the multiscale simulations have the potential to become a valuable tool across a range of emerging areas in bioengineering, such as understanding and predicting diseases and elucidating complex biological processes.

## 3 Macroscale

### 3.1 Study 5 - Micro to Macroscale simplification and simulation of a vertebral body

As shown previously, micro and mesoscale approaches are suitable for computational simulation and can provide highly complex mathematical models to fit the related real scale behavior. In the macroscale field, things are not different. The conventional approach consists of using consolidated mathematical models, embedded in commercial finite element simulation software, in order to represent macroscopic multiphysics behavior.

To use this class of solution is mandatory to elaborate hypothesis and simplifications, in order to fit the reality to the proposed mathematical models. Despite the fact that this is a consolidated procedure and very well adapted for the use of computational simulation, this approach does not sustain itself when subjected to a multiscale bioengineering metamodeling approach, like the one proposed in this work.

Even if one adopts the current approach, based on conventional mechanical material properties characterization and the use of current mathematical material models implemented in most of the finite elements mechanical simulation software, it is possible to assume microscopic finite elements simulation to achieve microscale like results and translate them into macroscale behavior by applying homogenization techniques. A straightforward approach can be used assuming the heterogeneity of a small piece of material and fit the mechanical response of such complex condition to a simple hypothetical homogeneous material with similar geometry.

A similar approach was applied to this case study (figure 7), where a vertebral body, composed of thin cortical walls, isotropic material, and trabecular bulk volume, also considered as isotropic materials, was represented as a homogeneous bulk volume with only one similar isotropic material property.

The homogenization process consisted in producing a complex model, representing the vertebral body as a two material system composed of thin walls cortical bone filled with bulk volume trabecular bone, both considered isotropic materials with different characteristic material properties, identified by their particular Young's moduli and Poisson ratios. The solution for this model showed a mechanical behavior, characterized by numerical results in terms of nodal displacements and stress fields. In order to produce an equivalent mechanical behavior, the displacements must be similar, provided the stress fields must be different due to the difference from the heterogeneous to homogeneous material model homogenization. This was a manual iterative process where the comparison of the displacement results in the direction of the long axis of the spine of few nodes, located in the upper free surface of each vertebral body, the one where the intervertebral discs connect, led to variations when considering a hypothetically homogeneous isotropic material property. While the difference between de displacement results between the micro and macro models still higher than an error of 0.001, the isotropic material property of the macroscale model was changing. When the displacements response considered converged, what was achieveda similar isotropic material property for the macroscale model and it was used to run the Large Scale Model (figure 7).

### 3.2 Study 6 - Development of an Additive Manufactured Socket for Transtibial Amputee

In this study, a digitization software was used to acquire 3D geometry, both external anatomy of limb and internal bone using InVesalius open-source software (22). A 3D CAD software was used for editing the anatomical geometries and modeling of the socket (figure 8a), integrating it with the conventional mechanical parts for the complete prosthetic device. A Finite Elements Analysis software was used to simulate mechanical behavior, especially the mechanical pressure distribution of the contact between the internal socket surface and the skin of the remaining limb of the patient to keep the socket attached with maximum comfort for the patient.

The primary objectives of the mechanical analysis were to find the best geometrical configuration in order to achieve low and well distributed mechanical pressure along the contact surface of the remaining limb. In this case, the purpose of keeping socket well attached is to assure some concentrated pressure regions in order to promote better stability while in use. The result can be seen in figure 8b and c.

## Micro to macroscale simulation
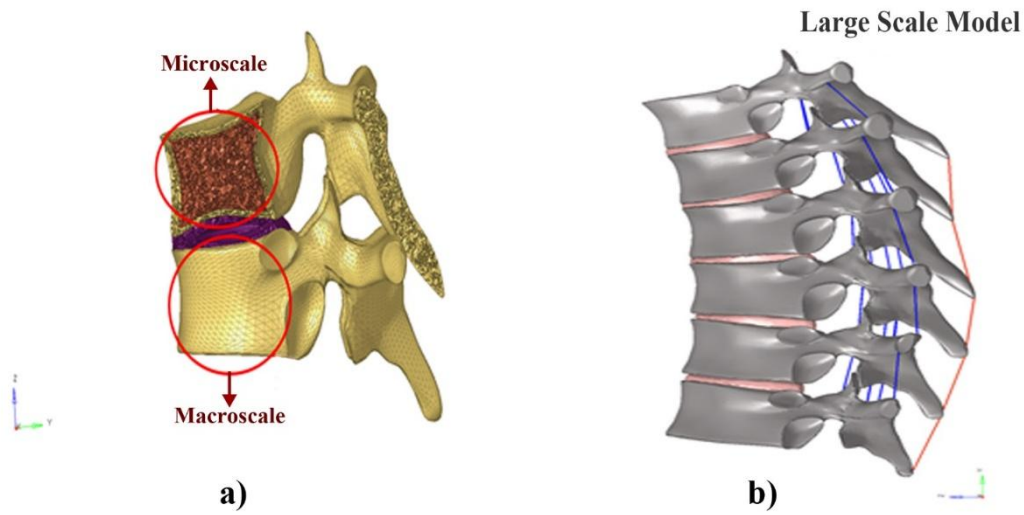


*Fig. 7a) microscale model of thevertebral body, with external cortical bone and internal trabecular bone,macroscale model of thevertebral body, with only one whole volume;b)large scale model that demanded a macroscale simplification.*

## Macroscale simulation between the socket and limb
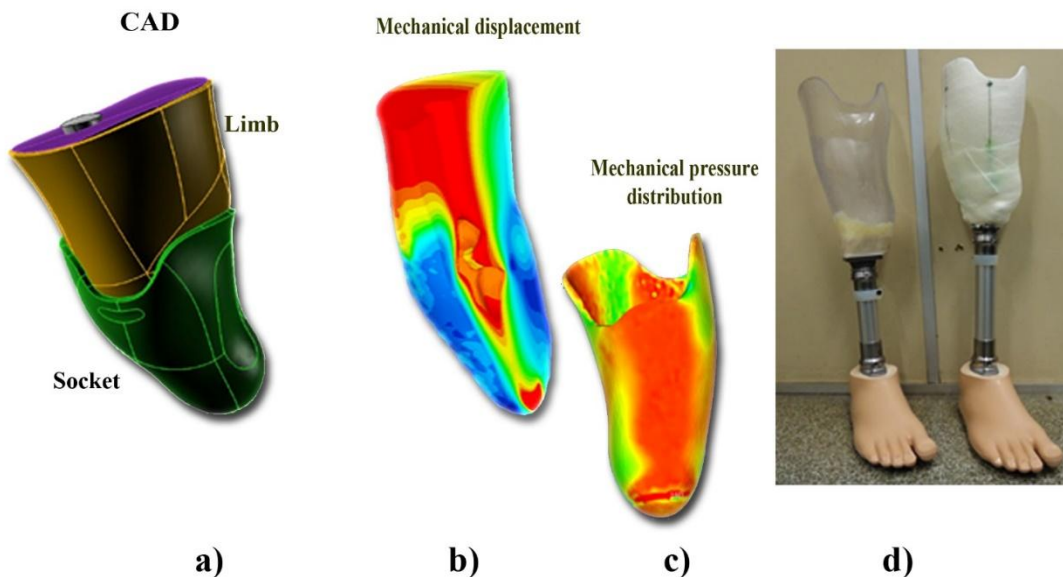


*Fig. 8 a) The complex system model for limb and socket for atranstibial prosthesis;b) Mechanical displacement result for biological structures; c) Mechanical pressure distribution for the socket; d)A prosthetic device made by conventional technique on left and additive technology on the right.*

The maximum Principal stress distribution in the socket (Figure 8c) was used to identify hot spots that could induce potential wounds to the patient's skin as well as to identify places where the pressure must be kept to achieve better fixation while avoiding wounds.

The designed socket was manufactured using additive manufacturing SLS (Selective Laser Sintering) technology in Polyamide thermoplastic polymer. After produced the socket was assembled to the rest of the parts provided by an Ottobock partnership (figure 8d) and tested in a real patient, showing that the use of such set of software is fully capable of offering high quality and applied results.

## 4 Conclusions

Computer simulation in bioengineering and for biological applications are not simple tasks. Many considerations and simplifications are necessary to establish a possible model that represents the necessary characteristics of the chemical, biochemical and physical phenomena. Those models can be classified into different scales ranging from molecules size to a whole organ and body. The complete integration of the multiscale models is of great importance for future research and applications bringing a better understanding of the whole process but still miss a framework capable of exchange data and information among such stand-alone models.

## Acknowledgements

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