

# NUMERICAL CALCULATIONS IN TISSUE ENGINEERING

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**ABSTRACT:** The design of optimized scaffolds for tissue engineering is a key topic of research, as the complex macro- and micro- architectures required for a scaffold depends not only on the mechanical properties, but also on the physical and molecular queues of the surrounding tissue within the defect site. Thus, the prediction of optimal features for tissue engineering scaffolds is very important for its mechanical, vascular or topological properties. The relationship between high scaffold porosity and high mechanical properties is contradictory, as it becomes even more complex due to the scaffold degradation process. A scaffold design strategy was developed, based on the finite element method, to optimise the scaffold design regarding the mechanical and vascular properties as a function of porosity. Scaffolds can be considered as a LEGO structure formed by an association of small elementary units or blocks. In this research work, two types of family elementary scaffold units were considered: non-triple periodic minimal surfaces and triple periodic minimal surfaces that describe natural existing surfaces. The main objective of this work is to present the undergoing research based on numerical simulations for the evaluation and prediction of the scaffold's behaviour under structural and vascular loading, and its topological optimisation.

## INTRODUCTION

Today, its incidence in modern medicine is growing with the ageing of the population and higher expectations for a better quality of life, together with the increasing number of required implants and organ shortage. The loss or failure of an organ or tissue is a frequent, devastating, and costly problem in health care. Currently, this problem is treated either by transplanting organs from one individual to another or performing surgical reconstructions, transferring tissue from one location in the human body into the diseased site. The need for substitutes to replace or repair tissues or organs due to disease, trauma, or congenital problems is overwhelming. Tissue engineering emerged to overcome these limitations by creating cell-based substitutes of native tissues (Risbud, 2001).

Tissue engineering is a multidisciplinary field focusing on the use of cells and engineered materials, combining the principles of biology, engineering and medicine to create biological substitutes for lost or defective native tissues (Eshraghi and Das, 2010). According to Skalak and Fox (1988), tissue engineering can be described as “the application of the principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue and organ functions” (Bártolo et al, 2008). This new research domain emerged as a rapidly expanding approach to address the organ shortage problem and comprises tissue regeneration, organ substitution and gene therapy. Diseases, such as Parkinson, Alzheimer, osteoporosis, spine injuries or cancer, might in the near future be treated with methods aiming at regenerating diseased or damaged tissues.

Three general strategies were adopted for the creation of new tissues (Bártolo et al, 2008):

- Cell-based strategies, involves the direct in vivo implantation of isolated cells or cell substitutes and it is based on cells synthesizing their own extracellular matrix (ECM). This approach avoids surgery complications allowing replacing only the damaged cells required for a specific function. The main limitations include immunological rejection and failure of the encapsulated cells.
- Growth-factor-based strategies, based on the use of growth factors and controlled-released systems. Growth factors are proteins secreted by several cell types functioning as signalling molecules, promoting cell adhesion, proliferation, migration and differentiation. The success of this strategy depends on the growth factors and controlled released systems.
- Scaffold-based methods, based on the use of a temporary scaffold, provide a substrate for the implanted cells and a physical support for tissue formation in an organized way. In this approach, transplanted cells adhere to the scaffold, proliferate, secrete their own ECM and stimulate new tissue formation. This strategy involves either a closed or an open system. In a closed system, cells are isolated from the body by a permeable membrane that allows exchange of nutrients and wastes, and protects cells from the immune response of the body. An open system begins with the in vitro culture of cells, which are then seeded onto a scaffold. The cell-matrix construct is then implanted into the body.

The cell-seeded temporary scaffold approach is the most widely used strategy for tissue engineering (Fuchs et al, 2001). In this approach, living cells are obtained from a tissue harvest, from either the patient (auto-graft) or a different person (allograft), and cultured in vitro on a three-dimensional biodegradable scaffold to obtain a tissue construct suitable for transplantation (Liu and Czernuszka, 2006). Scaffolds provide an initial biochemical substrate so that the novel tissue can grow until cells produce their own ECM. Therefore, scaffolds not only define the 3D space for the formation of new tissues, but also serve as temporary skeletons to accommodate and stimulate new tissue growth with appropriate functions (Bártolo et al, 2012).

The design of optimised scaffolds for tissue engineering is a key topic of research, as the complex macro- and micro- architectures required for a scaffold depends on the mechanical properties, physical and molecular queues of the surrounding tissue within the defect site. Thus, the prediction of the optimal properties for tissue engineering scaffolds is very important, for mechanical, vascular or topological properties.

The relationship between high scaffold porosity and high mechanical properties is contradictory as it becomes even more complex due to the degradation process of the scaffolds. A scaffold design strategy based on the finite element method was developed in order to optimise the scaffold design, regarding mechanical and vascular properties as a function of porosity. Scaffolds are considered as a LEGO structure formed by an association of small elementary units or blocks. In this research work, two types of family elementary scaffold units were considered: non-triple periodic minimal surfaces and triple periodic minimal surfaces that describe natural existing surfaces. The main objective of this work is to present the undergoing research based on numerical simulations for the evaluation and prediction of the scaffold's behaviour under structural and vascular loading, and its topological optimisation.

## **SCAFFOLD NUMERICAL MODELLING FOR TISSUE ENGINEERING**

The prediction of the effective optimal properties of tissue scaffolds is very important for tissue engineering applications, either regarding mechanical, vascular or topological properties (Almeida et al, 2007). To aid this specific issue of the design process of scaffolds, a computational tool,

Computer-Aided Design of Scaffolds (CADS) (Almeida and Bártolo, 2008), is been developed which enables to quantify the structural heterogeneity and mechanical and vascular properties of a scaffold with a designed macro and microstructure (Bártolo et al, 2009). Besides these topics, this computational tool already contemplates other issues such as degradation.

Computer-Aided Design of Scaffolds (CADS) makes the bridge between different computer applications such as CAD modelling systems and different numerical simulation tools for structural, topological and fluid dynamics optimisation based on the Finite Element Method. Three different numerical simulations may be used in scaffold design: a) structural and b) vascular evaluation of the scaffold's performance, and c) a topological optimisation of a scaffold for a specific structural application. The three numerical domains will be discussed below in further detail.

### STRUCTURAL ANALYSIS

The main goal for simulating the scaffold's mechanical behaviour is to evaluate the porosity dependence on the elastic modulus, where a tensile solicitation was applied to the geometric models. In order to validate the proposed approach, a compression solicitation was also applied to non-triple periodic surfaces and the obtained results were compared with experimental data. From a mechanical point of view, it is possible to observe that (Almeida, 2013):

- By increasing the porosity, the mechanical properties decrease in a non-linear way.
- In order to validate the proposed mechanical simulations, a comparison between numerical and experimental data was performed (fig. 1). In this case, a crushable foam behaviour was adopted for the numerical simulations. This comparison was undertaken for three pore sizes (450  $\mu\text{m}$ , 550  $\mu\text{m}$  and 650  $\mu\text{m}$ ). Results demonstrate that the scaffold with a pore size of 450  $\mu\text{m}$  presents the best numerical results when compared to the experimental compressive data. The plots from the numerical simulations illustrate that an unbalanced meshed body results in an unbalanced bulging phenomenon, which influences the densification process. This phenomenon can justify the deviations verified between the numerical stress-strain curves and the experimental ones. In order to obtain a better agreement between the numerical stress-strain curves and the experimental ones, the numerical parameters of all three structures need to be optimised and a symmetric meshed body needs to be defined.

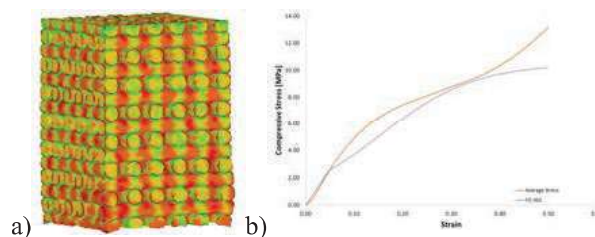


Figure 1. a) Numerical plot of the tensile stresses of a scaffold under compression. b) Comparison between the numerical and experimental compression.

## VASCULAR ANALYSIS

The main goal for simulating the scaffold vascular behaviour is to evaluate the porosity dependence on both Wall Shear Stress and Shear Stress Rates on the surface of the scaffold. These two parameters are the most critical due to the fact that they contribute to the cell's performance regarding their proliferation and differentiation along the scaffold structure internally and externally. From the results, it is possible to observe that (Almeida, 2013):

- For the scaffold structures with pore sizes of 450  $\mu\text{m}$ , 550  $\mu\text{m}$  and 650  $\mu\text{m}$ , both parameters present a similar behaviour. Results demonstrate that as the pore size increases, the difference is lower in value for the Shear Strain rate, and the Wall Shear Stress between the outer and inner regions of the scaffold, due to the decrease in the difficulty of the fluid to flow to the interior of the scaffold.
- For the Shear Strain Rate of Triple periodic minimal surfaces, it is possible to observe that the Shear Strain Rate decreases with the geometries' thickness and increases with the radius, for the Schoen surfaces. The Schwartz geometries have a similar behaviour for the Shear Strain Rate. In conclusion, the best geometric option is the scaffold with lower thickness and higher radius values.
- For the Wall Shear Stress of Triple periodic minimal surfaces, it is possible to observe that the Wall Shear Stress increases for the Schwartz geometry and decreases for the Schoen geometries, with the increase in thickness. The geometric variation regarding the radius variation has a similar behaviour compared to the thickness.
- Regarding the Wall Shear Stress of Triple periodic minimal surfaces on a micro-scale (fig. 2), the Schoen geometries present a more homogenous variation with lower thickness values, for both interior and exterior regions of the scaffold. The most significant variation is presented with the thickness variation of the Schwartz geometries, so the Wall Shear Stress increases with the increase of thickness maintaining a homogenous distribution on the exterior region of the scaffold. Regarding the interior region of the scaffold, it tends to decrease with a homogenous distribution.

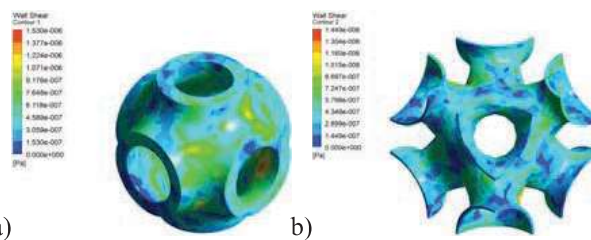


Figure 2. Wall shear stress of a) Schwartz and b) Schoen geometric scaffold.

## TOPOLOGICAL OPTIMIZATIONS

Topological optimisation provides the first design concept of the structure's materials distribution. Its goal is to minimise the structure compliance while satisfying the constraints of volume removal. As the structure compliance is twice the strain energy, the objective function of minimising structure compliance is equivalent to minimising strain energy. Regarding the topological optimizations, two types of analysis were carried out (Almeida, 2013):

- Topological optimisation of an initial solid block under different constraints (fig. 3.a):  
In this analysis, several scenarios were considered, combining loading and constraint conditions on vertices, edges and faces. In this case, two types of design models were obtained, invalid and valid scaffold models. Design models that present a shell structure or non-interconnected designs are considered invalid scaffold models, while design models that present interconnectivity in 1, 2 or 3 directions are considered valid scaffold models. From the results, it is possible to conclude that the best topological scenario corresponds to a scenario with constraints on the edges in 1 direction and displacements on the edges in the other 2 directions, obtaining 7 valid scaffold design models with interconnectivity in all 3 directions.
- Topological optimisation of an initial solid block with loading and constraint surfaces defined based on geometrical information obtained from real biological tissues (Bio-surface constrained optimisation of a solid block element) (fig. 3.b):  
The initial external topology of the  $\mu$ CT data, which corresponds to a structure of a well-defined mechanical behaviour and porosity level, is the starting point of an optimisation scheme that enables to obtain constructs with different levels of porosity and mechanical properties, according to the required applications, based on biomimetic external surfaces. This particular topological optimisation scheme uses the surface boundaries to produce novel models with different characteristics, which are different from the initial  $\mu$ CT models. This approach enables to produce valid biomimetic scaffold topologies for tissue engineering applications.

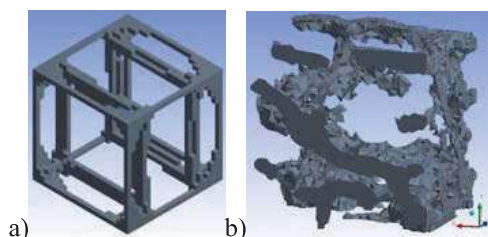


Figure 3. Topological optimization of a) initial solid block scaffold (90% porosity) and b) bio-inspired  $\mu$ CT-based scaffold (86% porosity).

## CONCLUSIONS

Scaffolds are critical elements for tissue engineering applications, as they provide the necessary support for tissue regeneration in an organised way. As described before, scaffolds should be biocompatible, biodegradable, with appropriate porosity, pore structure and pore distribution, on top of presenting both surface and structural compatibility. These characteristics are usually difficult to achieve, i.e., it is quite difficult to design a construct with both high porosity (a critical property for vascularisation purposes) and high mechanical properties. Design strategies to produce optimized scaffolds represent a critical topic of research in the field of tissue engineering. This is an emergent domain covered by this research study.

The state-of-the-art in tissue engineering and scaffold design, based on numerical simulations to determine optimum scaffold design parameters, is fully addressed. The knowledge gathered through this research work was fundamental to develop a computational strategy for the design of tissue engineering scaffolds. Reviews of computer-aided design of scaffolds in tissue engineering can be found in “Smart Design of Scaffolds Obtained by Biofabrication for Tissue Engineering

Applications” (Almeida, 2013), “Structural and Vascular Analysis of Tissue Engineering Scaffolds: Part 1 – Numerical Fluid Analysis” (Almeida and Bártolo, 2012a), “Structural and Vascular Analysis of Tissue Engineering Scaffolds: Part 2 – Topology Optimization” (Almeida and Bártolo, 2012b) and “Computer-Aided Tissue Engineering” (Liebschner, 2012).

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