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ADOPTING GENETIC ALGORITHMS FOR OPTIMIZING SCAFFOLDS IN ALGINATE FOR BIOFABRICATION

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Alternative ways of restoring and replacing tissues have been researched and implemented successfully against the increase of the rate of transplants due to damaged or affected tissues or organs by accidents or diseases and also by the aging of the population in many countries. Biofabrication by means of Rapid Prototyping techniques can help in the fashioning and final production of scaffolds devoted to support and stimulate the growth of new tissues. For soft tissues, a biomaterial known as Alginate has been studied and used as raw-material for scaffolds fabrication. A Scaffold should own very dynamical and adaptive characteristics. In this sense, it is fundamental to know better the mechanical and chemical properties since the scaffold must guarantee good strength and stiffness at the same time the material degrades gradually. The present and future of biomedical materials development requires this degree of control prediction in the design, synthesis, and function of next-generation materials. A prediction job is possible and it has already been used so that the scaffold state can be forecasted before its fabrication and, as a good alternative, to know how and how much alginate should be used. A single mathematical model experimentally obtained describes an interesting physical behaviour, that is, in the case of this work, the degradation of alginated-scaffolds. Evolutionary algorithms, like Genetic Algorithms (GAs), represent a class of stochastic optimization procedures based on natural systems according to Darwin's observations, and the modern synthetic theory of evolution. In the present work, the objective of GAs is to find out the best values of alginate amount and initial porosity for scaffold fabrication that maximize the elastic modulus. In summary, the paper presents an optimization process scheme using Genetic Algorithms to maximize the elastic modulus and therefore to aid the design of scaffolds in alginate. The optimization is very welcome to Tissue Engineering and Biofabrication.

1. INTRODUCTION

Biofabrication can be defined as the production of complex living and non-living biological products from raw materials such as living cells, molecules, extracellular matrices, and biomaterials. A scaffold, as an extracellular matrix, is a temporary supporting structure. There are synthetic and naturally derived solid scaffolds (Mironov, 2009). Biofabrication uses cells or biologics as the basic building blocks in which biological models, systems, devices and products are manufactured (Sun, 2009). Biofabrication links the Tissue Engineering and the Rapid Prototyping technologies. 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 function. Rapid Prototyping helps in the fashioning and final production of scaffolds devoted to support and stimulate the growth of new tissues. For soft tissues, a biomaterial known as Alginate has been studied and used as raw-material for scaffolds fabrication (Fundueanu *et al.*, 1999). A scaffold must own very dynamical and adaptive characteristics in order to be implanted and to take its main roles which are to carry the stem live cells inside it, to back the growth of these cells and besides this to biodegradate appropriately since the minimum material

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should remain after the tissue is reconstructed (Rezende *et al.*, 2009). It is fundamental to be aware of the mechanical and chemical properties since the scaffold must guarantee good strength and stiffness at the same time the material degrades gradually. In this way, the optimization process comes, supported by Genetic Algorithms, to maximize the elastic modulus and therefore to aid the design of scaffolds in alginate.

To know how the mechanical behaviour of the scaffold will be, some time later, is the keyword. And the understanding about the match between biodegradation and Young Modulus is mandatory.

The present and future of biomedical materials development requires this degree of control prediction in the design, synthesis, and function of next-generation materials (Hutmacher, 2006). A prediction job is possible and it has already been used so that the scaffold state can be forecasted before its fabrication and, as a good alternative, to know how and how much alginate should be used. Other future analyses can be around the best geometry to be adopted during Rapid Prototyping technique actuation.

2. SCAFFOLDS

The function of a degradable scaffold is to act as a temporary support matrix for transplanted or host cells so as to restore, maintain, or improve tissue. A scaffold, as shown in Figure 1, may be created from various types of materials, including polymers. Polymeric scaffolds may be used to support a variety of cells for numerous tissues within the body. The design of a polymeric scaffold plays a significant role in proper cell growth. Therefore, several important properties must be considered: fabrication, structure, biocompatibility, biodegradability, and mechanical strength, as illustrated in Figure 2. Scaffolds guide cells to grow, synthesize extracellular matrix and other biological molecules and facilitate the formation of functional tissues and organs (Ma, 2004; Zhang and Ma, 2000).

The scaffold is expected to support cell colonization, migration, growth and differentiation, and to guide the development of the required tissue or to act as a drug delivery device (Hutmacher, 2006).

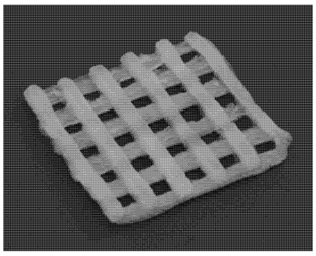


Figure 1: Example of a handcraft scaffold.

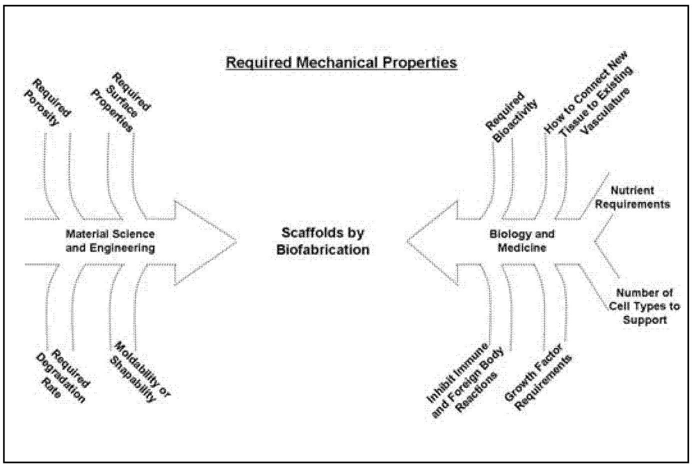


Figure 2: Requirements of a scaffold for usage in Tissue Engineering.

3. ALGINATES

Alginate is an anionic copolymer composed (Figure 3) of homopolymeric regions of 1,4-linked β-D-mannuronic (M blocks) and α-L-guluronic acid (G blocks), interspersed with regions of alternating structure. The industrial manufacture of alginate is based on the extraction of a polymer from brown algae. Gelation occurs when divalent ions (Ca2+, Ba2+, Fe2+, Sr2+, etc.) or trivalent ions (Al3+, etc.) take part in the interchain ionic binding between G-blocks in the polymer chain giving rise to a three dimensional network. Such binding zones between the G-blocks are often referred to as “egg boxes”. These ions act as cross-linkers that stabilise alginate chains forming a gel structure, which contains cross-linked chains interspersed with more freely movable chains that bind and entrap large quantities of water.

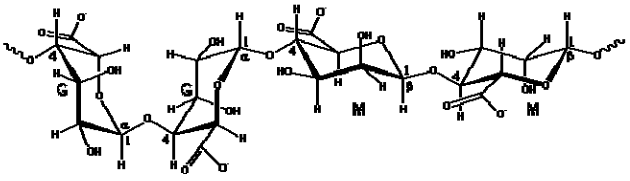
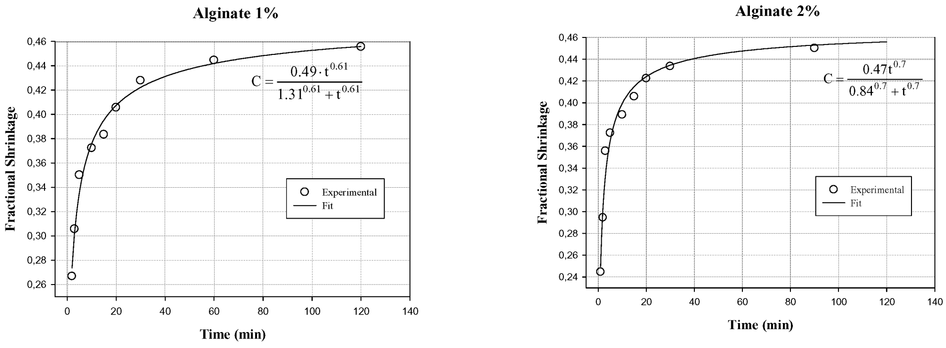


Figure 3: Structure of alginate.

3.1 Alginate Shrinkage

A match between the alginate shrinkage in terms of time was obtained experimentally for three different proportions of alginate: 1%, 2% e 5%, and got through the mixture of solutions with 1g, 2g and 5g of alginate, respectively, in 100 ml of water with a $CaCl_2$ 0,3 Molar solution (Rezende *et al.*, 2007). All solutions were prepared with pure water, with conductivity of 0.054 µS/cm. Alginate solutions were prepared by addition of weighted portions of sodium alginate to measured volumes of water. Due to their high viscosity, these solutions were agitated by orbital shaking for three hours at 50 ºC to ensure good homogeneity. Calcium chloride solution 5% (w/v) was obtained dissolving the salt in water. This solution was diluted to obtain solutions containing different concentrations of calcium chloride. Sodium alginate was purchased at Panreac (Barcelona, Spain). Calcium chloride was supplied by Carlo Erba (Milano, Italy).

The mechanical properties vary along time due to degradation and porosity changes. The degradation of alginate structures was determined through the analysis of the shrinkage variation along time as shown in Figure 4 (Rezende *et al.*, 2008).



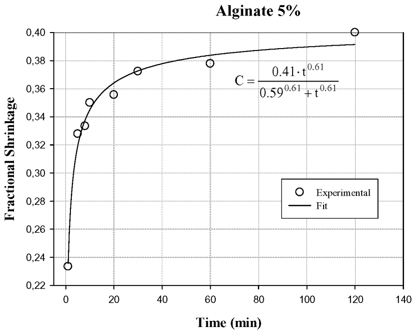


Figure 4: Fractional Shrinkage in terms of alginate concentration.

4. THE GENETIC ALGORITHMS (GAs)

Evolutionary algorithms, like Genetic Algorithms (GAs), represent a class of stochastic optimization procedures based on natural systems according to Darwin's observations, and the modern synthetic theory of evolution. The Genetic Algorithms approach starts with a random population of chromosomes that are a set of solutions for the optimization problem. Traditionally, solutions are represented in binary as strings of algorisms 0 and 1, but a real encoding is also possible. In each generation, the fitness of every individual in the population is evaluated, multiple individuals are stochastically selected from the current population (based on their fitness), and modified (recombined and possibly randomly mutated) to form a new population. The new population is then used in the next generation. Usually, the algorithm terminates when either a maximum number of generations has been reached, or a satisfactory fitness level has been found for the population.

5. MODELLING DOR THE OPTIMIZATION

The optimization problem is to determine optimal features for the fabrication of optimized alginate scaffolds for Tissue Engineering. The optimization goal aims at finding optimal values of alginate composition and initial porosity in order to fabricate scaffolds with, at a pre-determined time, a maximal mechanical behaviour (elastic modulus).

The optimization problem is given by (Rezende *et al.*, 2007):

$$\underset{[\alpha,\phi_0]}{\text{Maximize}} \quad E(\phi_0,\alpha,t)$$

$$\text{Subject to:} \quad 1\% \leq \alpha \leq 8\% \qquad 30\% \leq \phi_0 \leq 80\% \qquad \textbf{(1)}$$

where E is the elastic modulus (shear effects are not considered), α is the alginate composition and ϕ_0 is the initial porosity.

It is important to emphasize that after certain period of time, the natural biomaterial degradation reduces the mechanical properties of the scaffold, that is, reduces the Young Modulus. In this sense, the objective is to determine which are the optimum values of the pair [alginate composition (%alg), initial porosity (ϕ_0)] for the fabrication of a scaffold that, after some long time, assure the objective function optimization given by the maximization of the Young Modulus. According to the values of this pair at scaffold fabrication, different values of Young Modulus can be achieved.

The shrinkage process can be modeled through a three parameters sigmoidal model given by:

$$C(\alpha,t) = \frac{\varsigma(\alpha)\cdot t^{\vartheta(\alpha)}}{\lambda^{\vartheta(\alpha)} + t^{\vartheta(\alpha)}} \tag{2}$$

where t is the time and $\varsigma, \vartheta, \lambda$ are variables that depend on the alginate composition (α). Porosity at each time is also a function of alginate composition and shrinkage:

$$\phi(\phi_0,\alpha,t) = \phi_0 + \zeta(\phi_0,\alpha)\cdot C(\phi_0,\alpha,t) + \psi(\phi_0,\alpha)\cdot C^2(\phi_0,\alpha,t) \tag{3}$$

where ζ, ψ are constants depending on alginate composition and C is the shrinkage.

The Young Modulus E is given by an expression that reports straightly the initial Young Modulus and the final porosity of the scaffold and indirectly the pair [alginate composition, initial porosity]. The dependence between the elastic modulus and porosity for different alginate compositions is given by the following equation:

$$E(\phi_0,\alpha,t) = E_0(\phi_0,\alpha) + k_1(\phi_0,\alpha)\cdot\phi(\phi_0,\alpha,t) + k_2(\phi_0,\alpha)\cdot\phi(\phi_0,\alpha,t)^2 + k_3(\phi_0,\alpha)\cdot\phi(\phi_0,\alpha,t)^3 \tag{4}$$

with E_0 being the initial elastic modulus, k_1, k_2, k_3 constants dependent on both the alginate composition and the initial porosity and ϕ the final porosity of the scaffold.

A single constrained optimization problem, which maximises the elastic modulus, constraints considers four cases of constraints at shrinkage and final porosity: 1) no constraint, 2) shrinkage higher than 25%, 3) final porosity higher than 80% and 4) Shrinkage < 35% and Final Porosity > 75%.

To solve the constrained problem, a constraint handling method based on the penalty function approach was used, not requiring any penalty parameter (Deb, 2000). In this case, the expression of the fitness function for a minimisation problem, where infeasible solutions are compared based only on their constraint violation, is given by Deb (2000):

$$F(\mathbf{x}) = \begin{cases} f(\mathbf{x}) & \text{if } g_j(\mathbf{x}) \geq 0 \quad \forall j=1,2,\ldots,nc \\ f_{\max} + \sum_{j=1}^{m} \langle g_j(\mathbf{x}) \rangle & \text{otherwise} \end{cases} \tag{5}$$

where $f_{\max}$ is the objective function value of the worst feasible solution in the population.

The GAs used in this research work, to solve the formulation indicated above in section 3, are a Fortran binary code (Carroll, 2008). The employed genetic operators are the tournament selection, the uniform crossover, the creep and the jump mutation. Niching and elitism are also employed. The input parameters, chosen by a trial and error method, are indicated in Table 1.

Table 1: The GAs input parameters.

GAs input parameters	Value
Population size per generation	50
Maximum number of generations	30
Crossover probability	0.60
Jump mutation probability	0.077
Creep mutation probability	0.077
Initial random number seed for the GAs run	-1000

6. ANALYSIS OF THE SCAFFOLD OPTIMIZATION USING GAs

The analyses were shared in four cases including the first one with no constraint and the remaining cases constrained.

6.1 Constraint Considerations

Four different cases with respective conditions were evaluated. The single objective constrained optimization problem which maximises the elastic modulus considers one case with no constraint and three cases of constraints at shrinkage and final porosity (Table 2).

Table 2: The Four Cases Analyzed.

Cases	Conditions
1	No constraint
2	Shrinkage > 25%
3	Final Porosity > 80%
4	Shrinkage < 35% and Final Porosity > 75%

6.2 Results

This section presents the results of the scaffolds optimization using Genetic Algorithms for the constrained and unconstrained problem.

a) No constraint

Results obtained for this case are shown in Table 3:

Table 3: Optimization results for the constrained problem (no constraint)

Optimization Variables	Initial Alginate composition (%)	8,00
	Initial Porosity (%)	30,00
Objective Function	Elastic modulus (KPa)	23,38
Constraint	Shrinkage (%)	16,24
Output Variable	Final Porosity (%)	59,17

Figure 5 shows the evolution of the objective function along all the generations. Profiles of the objective function, shrinkage and final porosity are illustrated in Figure 6. As can be seen through these figures, for the Young Modulus with no constraint the alginate composition trends to values close to the superior edge, while that for initial porosity the tendency is close to inferior limit.

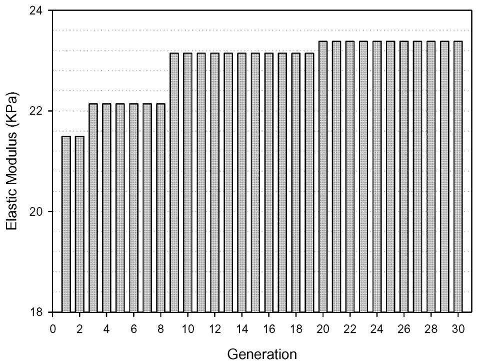


Figure 5: Young Modulus through generations (No Constraint).

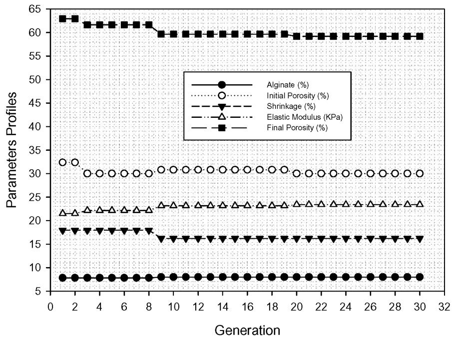


Figure 6: Profiles after all the generations (No Constraint).

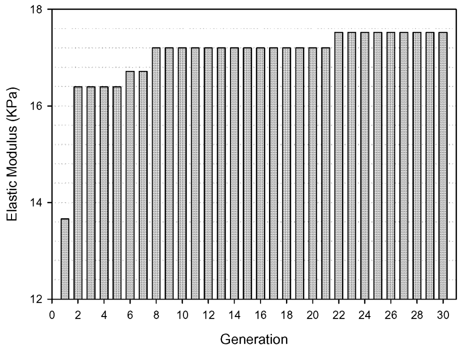
b) Constraint 1: Shrinkage higher than 25%

Results obtained for this case are shown in Table 4:

Table 4: Optimization results for the constrained problem (shrinkage > 25%)

Optimization Variables	Initial Alginate composition (%)	7.06
	Initial Porosity (%)	30.00
Objective Function	Elastic modulus (KPa)	17.52
Constraint	Shrinkage (%)	25.22
Output Variable	Final Porosity (%)	70.97

Figure 7 shows the evolution of the objective function along all the 30 generations. In Figure 8, the profiles of the objective function, shrinkage and final porosity are also presented.



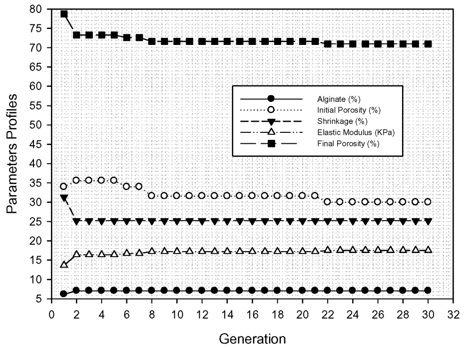


Figure 7: Young Modulus through generations (Shrinkage>25%).

Figure 8: Profiles after all the generations (Shrinkage>25%).

In order to the Young Modulus have a maximum value without violation of the constraint (shrinkage > 25%), alginate composition should be close to 7.06% and the initial porosity close to 30%.

c) Constraint 2: Final porosity higher than 80%

Results obtained for this case are shown in Table 5:

Table 5: Optimization results for the constrained problem (final porosity > 80%).

Optimization Variables	Initial Alginate composition (%)	5.79
	Initial Porosity (%)	32.38
Objective Function	Elastic modulus (KPa)	12.99
Output Variable	Final Porosity (%)	80.01
Constraint	Shrinkage (%)	33.29

Figure 9 presents how the objective function progresses along the generations. Profiles of the objective function, shrinkage and final porosity are shown in Figure 10.

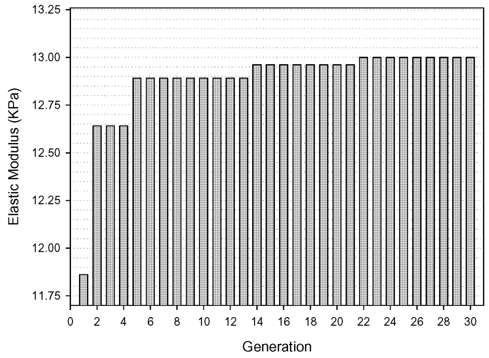


Figure 9: Young Modulus through generations (Final Porosity>80%).

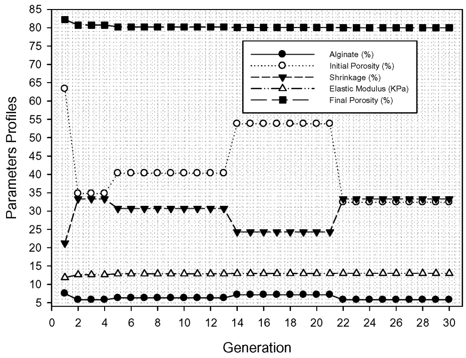


Figure 10: Profiles after all the generations (Final Porosity>80%).

The alginate composition should be close to 5.79% and the initial porosity close to 32.38%, in order that the Young Modulus reach a maximum value without violation of the constraint (shrinkage > 25%).

d) Constraint 3: Shrinkage < 35% and Final Porosity > 75%

Results obtained for this case are shown in Table 6:

Table 6: Optimization results for the constrained problem (Shrinkage < 35% and Final Porosity > 75%).

Optimization Variables	Initial Alginate composition (%)	7,45
	Initial Porosity (%)	47,46
Objective Function	Elastic modulus (KPa)	15,51
Output Variable	Final Porosity (%)	21,78
Constraint	Shrinkage (%)	75,03

Figure 11 reports the evolution of the objective function along the generations and Figure 12 illustrates the profiles of the objective function, shrinkage and final porosity.

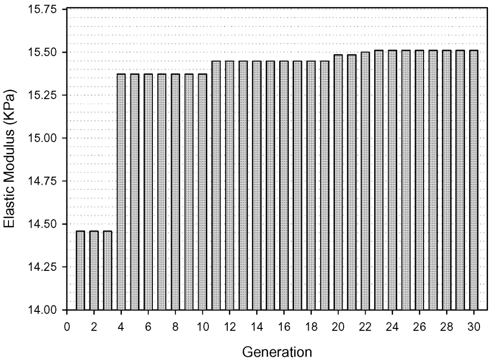


Figure 11: Young Modulus through generations (Shrinkage < 35% and Final Porosity > 75%).

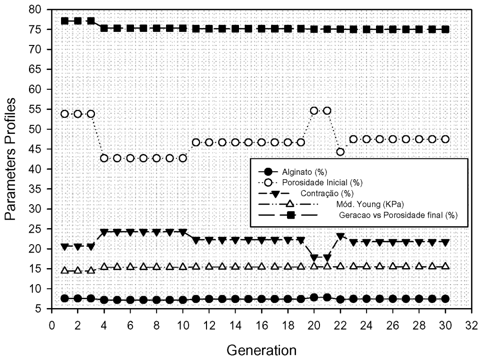
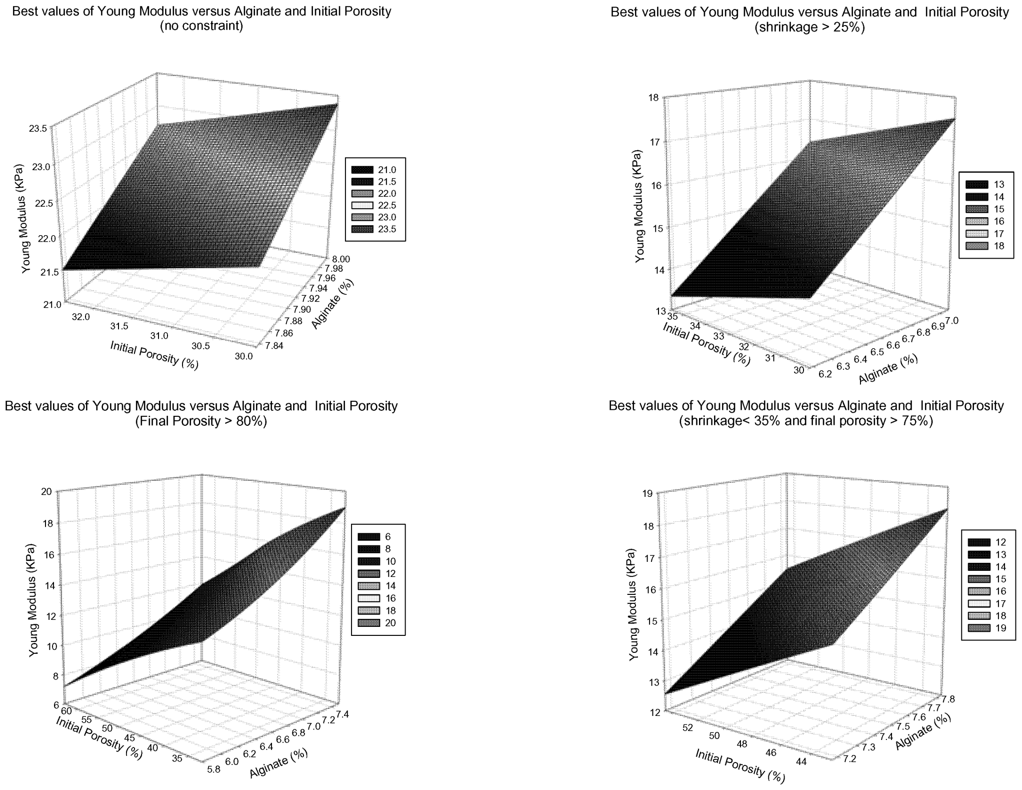


Figure 12: Profiles after all the generations (Shrinkage < 35% and Final Porosity > 75%).

For the maximal fitness (Young Modulus) without violation of the constraint (shrinkage > 25%), alginate composition should be close to 7.45% and the initial porosity close to 47.46%.

6.3 Visual summary of the results

As a summary of the best values obtained after the Gas run, Figure 13 illustrates in a 3D chart of the Objective Function (Young Modulus) and the Optimization Variables.


Best values of Young Modulus versus Alginate and Initial Porosity (no constraint)

Best values of Young Modulus versus Alginate and Initial Porosity (shrinkage > 25%)

Best values of Young Modulus versus Alginate and Initial Porosity (Final Porosity > 80%)

Best values of Young Modulus versus Alginate and Initial Porosity (shrinkage< 35% and final porosity > 75%)

Figure 13: A 3D graph of the best values for the Objective Function versus the Optimization Variables.

7. CONCLUSIONS

This research uses Genetic Algorithms to optimize the mechanical behaviour of alginate scaffolds for Tissue Engineering. The mathematical model was experimentally obtained and the values for both alginate composition and initial porosity of the scaffold were evaluated under different constraints being found that best values of the pair which maximizes the Young Modulus with no violation. The constrained maximization of the elastic modulus was determined through the optimization code. The next work is to extend this tool to perform topological optimization regarding other expressive effects like topology, for instance, integrating them with a broader simulation code.

8. ACKNOWLEDGEMENTS

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