COMPUTATIONAL MODEL OF FLOW AND BIOCHEMICAL TRANSPORT IN A MULTICHAMBER BIOREACTOR

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Introduction

Osteoarthritis (OA) is the most common clinical chronic joint disease with cartilage degeneration, damage and bone hyperplasia. Knee OA has an estimated prevalence between 12 % and 35 % in the general population and is considered the leading cause of musculoskeletal disability in the elderly population worldwide. **Mesenchymal stem cells** (MSCs) have attracted attention for clinical use because of their multilineage potential, limited immunogenicity, and relative ease of growth in culture. In this context, we aim at developing a combined approach consisting of a computational study and an algebraic model of a multichamber bioreactor, validated by experimental test. The former study is performed to evaluate quantitatively the degree of perfusion of nutrients in all of the regions of the bioreactor.

Materials and methods

Geometry and Mesh

The 3D CAD geometry of the bioreactor was created using **ANSYS ICEM CFD v.15.0** (Ansys Inc., Canonsburg, PA). The overall geometry is the repetition of four single unit cells, each formed by two separated chambers with the scaffold placed across them (Fig.1 A). The four single upper chambers are connected by an inlet line, the same for the bottom ones. Two domains were defined: fluid (grey in Fig.1 B) for the volume of the two chambers and porous (red in Fig.1 B) for the scaffold. Each computational domain is discretized with tetrahedral elements using the software ANSYS ICEM CFD adopting the *Octree Mesh Method*.

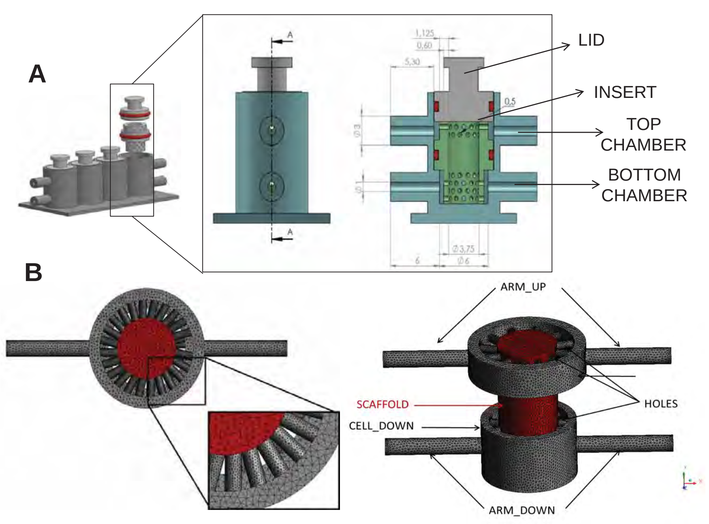

Fig.1 *A) CAD model (dimensions in mm) B) mesh.*

Computational Model

The computational analysis includes three sub-models:

1) **Fluid dynamic**: the fluid is considered isotropic, Newtonian and incompressible and *Stokes* equations were solved (*Re*<<0.1)
2) **Biomolecules advection:** nutrients supply (e.g. O_2, glucose, BSA) was implemented along a cell culture. Axial diffusion was omitted being *Pe*>1. All the simulations were performed with both scaffold in gelMa (photocrosslinkable methacrylated gelatin) and PLLA.
3) **Cellular metabolic consumption:** linear source term and Michaelis-Menten equation (non-linear).

A

biomolecule	Diffusion coefficient		
	fluid	gelMA	PLLA
Oxygen	2.1 x10^{-9} [m^2/s]	4.5 x10^{-10} [m^2/s]	0 [m^2/s]
Glucose	6.7 x10^{-10} [m^2/s]	3.958 x10^{-10} [m^2/s]	0 [m^2/s]
BSA	7.1 x10^{-11} [m^2/s]	3.918 x$^{10-18}$ [m^2/s]	0 [m^2/s]

Tab.1 *A) values of D for the advection analysis*

B

biomolecule	V_{max}	K_m
Oxygen	1.15 x10^{-17} [mol/cells s]	2.13 x10^{-4} [Kg/m^3]
Glucose	9.92 x10^{-17} [mol/cells s]	0.50 [Kg/m^3]

B) values for MM model

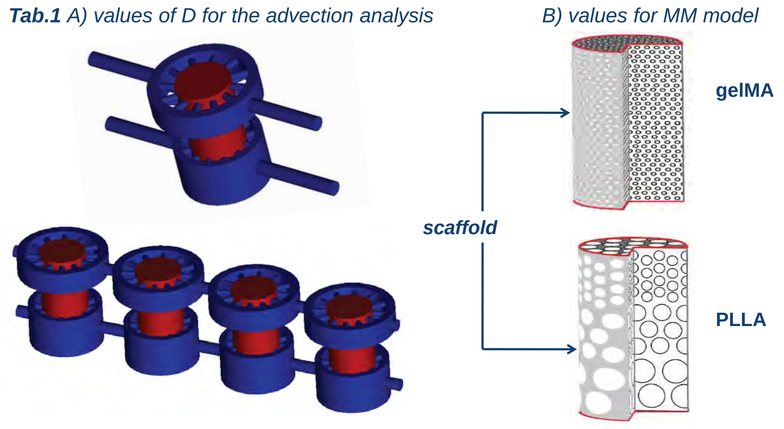

Fig.2 *Single and 4 array configurations (left) and sketch of the 2 types of scaffold (right).*

Algebraic Model

A purely algebraic tool was developed, using 2 ANSYS simulations, to obtain the output of a physical quantity in a known geometrical configuration. The problem is linear, so it can be solved using the linear operator M (matrix 2x2). M represents the input/output relationship of the geometrical quantity (e.g., flow) for a given bioreactor geometry.
Subsequently, the model was extended to n cells case and the overall I/O relationship was found. It was demonstrated that the operator I/O for n cells in a row is Mn, a combination of n operators of single cells.

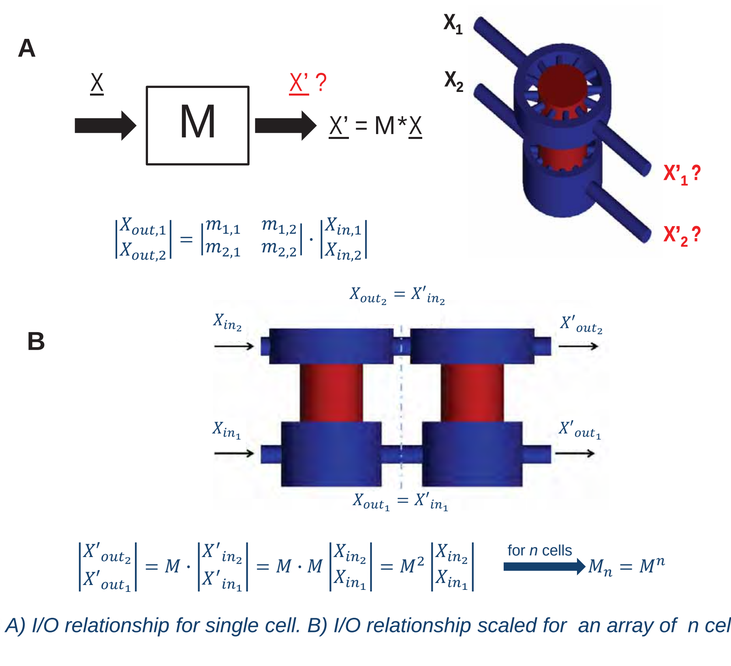

Fig.3 *A) I/O relationship for single cell. B) I/O relationship scaled for an array of n cells*

Experimental set up

- Culture media mix: spectrophotometric analysis.
- BSA concentration: spectrofluorometric analysis
- Cellular glucose consumption: spectrofluorometric analysis. GelMA scaffold seeded with 10^6 [cells / ml], PLLA scaffold with $8x10^5$ [cells / scaffold]
- Cell differentiation: histological examinations

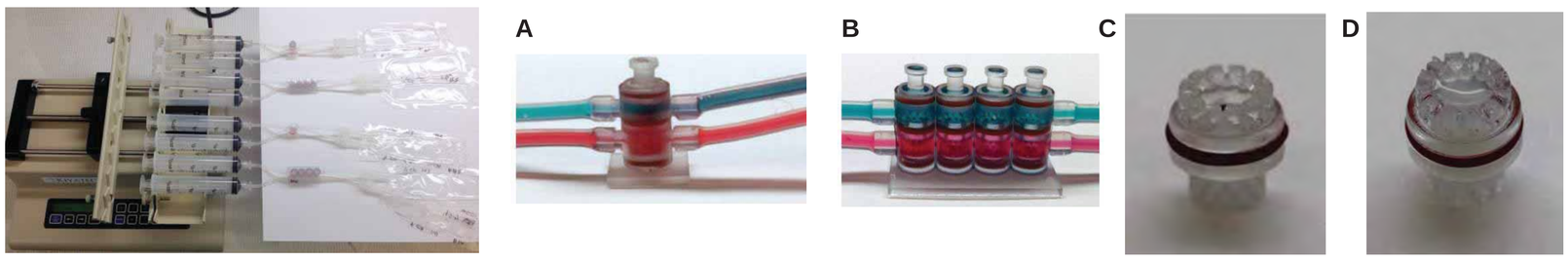

Fig.4 *Experimental setup: A) flux mix for one cell B) flux mix for 4-cells-array C) insert without scaffold D) insert with scaffold*

Results

Fluid dynamic analysis

In the presence of gelMA scaffold there is no significant communication between the two chambers media. This behavior is due to the low permeability of the gelatin, that shows a high resistance for the fluid flow that would be forced by the pressure gradient to migrate from one chamber to the other. For the PLLA scaffold, being not permeable for the fluid, there is no evidence in blocking the fluid moving from one chamber to the other. A higher mixing of culture media was observed with the highest peak in the case of flow gap in the inlets.

Biomolecules transport

A good match between simulations and experimental controls was found for all the configurations.
For glucose, oxygen and BSA gelMA offers the highest resistance to diffusion. On the contrary, PLLA impermeability to the molecules enhances the transport, but it is slightly lower than in the configuration with bioreactor without any scaffold. For sake of clarity, the comparison of numerical and experimental results are shown for the BSA concentration (Fig.6).

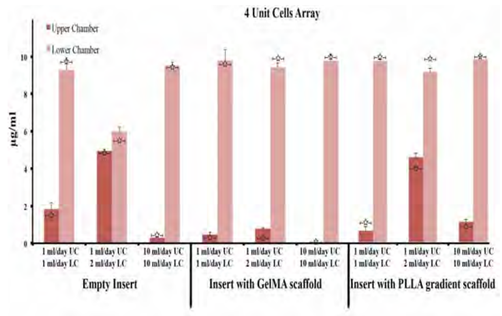

Fig.6 *Bars represent experimental data with standard deviation. Star columns correspond with numerical data*

Cellular consumption

The most percentage of glucose consumed in 4 cell array condition with PLLA scaffold respect than the gelMA one is due to the different cell density used in culture tests.

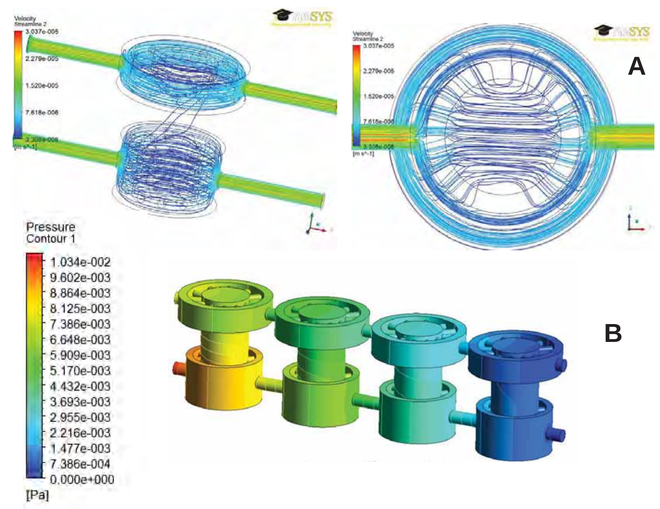

Fig.5 *A) flux stream lines B) mass flow distribution*

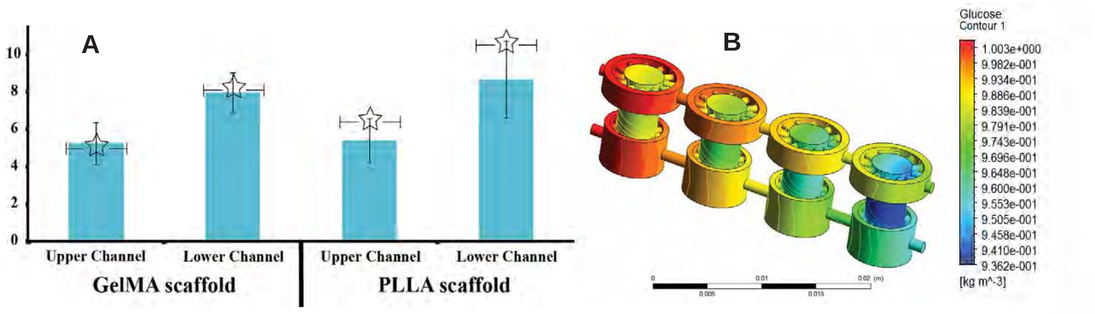

Fig.7 *A) glucose consumption: numerical vs experimental data B) consumption evolution in one cell and along the 4-cells-array*

Histologic analysis

From histological staining the differentiated phenotype (chondrogenic or osteogenic) is relative more evident in the case of PLLA scaffold than the gelMA one. Thus, cell differentiation and new osteochondral tissue formation is more consistent in PLLA presence. This achievement shows how numerical analysis are confirmed by experimental data.

Fig.8 *images taken at optical microscope Olympus. Histological staining: Alizarin Red and Alcian Blue.*

Conclusions

The ANSYS platform has proved successfully suitable to solve such problems. The developed model allows to use different scaffolds and different geometrical configurations. The microtissue culture system developed here offers novel capabilities for investigating the physiology of osteochondral tissue and pathogenic mechanisms of OA.

References

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