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Numerical optimization of cell colonization modelling inside scaffold for perfusion bioreactor: A multiscale model

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ABSTRACT

Part of clinically applicable bone graft substitutes are developed by using mechanical stimulation of flow-perfusion into cell-seeded scaffolds. The role of fluid flow is crucial in driving the nutrient to seeded cells and in stimulating cell colonization. A common numerical approach is to use a multiscale model to link some physical quantities (wall shear stress and inlet flow rate) that act at different scales. In this study, a multiscale model is developed in order to determine the optimal inlet flow rate to cultivate osteoblast-like cells seeded in a controlled macroporous biomaterial inside a perfusion bioreactor system. We focus particularly on the influence of Wall Shear Stress on cell colonization to predict cell colonization at the macroscale. Results obtained at the microscale are interpolated at the macroscale to determine the optimal flow rate. For a macroporous scaffold made of interconnected pores with pore diameters of above 350 μm and interconnection diameters of 150 μm, the model predicts a cell colonization of 325% after a 7-day-cell culture with a constant inlet flow rate of 0.69 $mL \cdot min^{-1}$. Furthermore, the strength of this protocol is the possibility to adapt it to most porous biomaterials and dynamic cell culture systems.

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1. Introduction

In the area of bone grafting, large bone deficits can arise from traumas, diseases, injuries or congenital defects. A bone graft substitutes is a scaffold in which cells are seeded. Cells need to grow to ensure a good densification of the grafts. Consequently, the bone graft substitutes need nutrients and waste removal. The static cell culture is the most common and the simplest way to develop bone graft substitutes[1–4]. However, the static cell culture has some drawbacks. First, the mechanical stimulation, an important parameter in cell growth [5,6], is neglected. Second, this method is not suitable for large bone substitute. Indeed, for large substitutes, nutrients cannot be delivered to the center of the scaffold and waste cannot be removed, leading to cell necrosis. One solution is the use of a device called a bioreactor that can generate dynamic cell culture [7–10]. This process provides both the necessary mechanical stimulation (pressure drop, shear stress) and the nutrient availability to cells [8,11]. There are numerous types of bioreactors. Direct perfusion bioreactors (DPB) efficiency is demonstrated in the enhancement of human cell growth and survival. These effects are mainly due to the constant renewal of the culture medium and the beneficial effect of fluids on cell activity [12–14].

Another important entity to study is the bone graft substitutes itself and its influence on cell culture. It would be unrealistic to give an exhaustive list of bone substitutes. In this paper, the study is limited to the case of a specific bioceramic. Due to the similar properties shared with human bones, bioceramics are nowadays widely used in bone grafting or in tissue engineering [15,16]. In particular, bioceramics based on calcium phosphate as hydroxyapatite (HA) or tricalcium phosphate (TCP) are highly developed due to their biocompatibility and osteoconductivity [17,18]. HA is used in this study for its law rate of biodegradation and therefore the stability of the macroporous structure of scaffold. An increasing number of studies provide a generous amount of details about their applications in bone grafting [19–23].

Cell culture takes place in a saturated bioceramic with culture medium. The biological fluid flows through the pores of the bioceramic material and induces a specific pressure on cells called Wall Shear Stress (WSS). This stress is a fundamental parameter affecting cell growth and survival [24]. In this study, wall shear stress is estimated on the walls of the scaffold. Wall Shear Stress depends on fluid velocity and this velocity is linked to the flow rate selected on the DPB. If the inlet flow rate is too low, mass transfer limitations cannot be overcome and nutrients cannot be present at the

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Nomenclature

Roman symbols

c_m	Michaelis-Menten constant ($mol \cdot m^{-3}$)
c_{O2}	Dioxygen concentration ($mol \cdot m^{-3}$)
d_i	Interconnection diameter (μm)
d_s	Pore diameter (μm)
D_x	Diffusion coefficient of x specie ($cm^2 \cdot s^{-1}$)
K	Permeability (m^2)
p or P	Pressure (Pa)
Q	Flow rate ($mL \cdot min^{-1}$)
Q_m	Max velocity of O_2 consumption ($mol \cdot cell^{-1} \cdot s^{-1}$)
R_c	Colonization rate (s^{-1})
R_d	Death rate (s^{-1})
u	Fluid velocity ($mm \cdot s^{-1}$)
u_D	Darcy velocity ($mm \cdot s^{-1}$)

Greek symbols

μ	Dynamic viscosity ($Pa \cdot s$)
ρ	Density of fluid ($kg \cdot m^3$)
ρ_{cell}	Cell Density inside a scaffold ($cell \cdot ml^{-1}$)
ρ_{max}	Maximum of ρ_{cell} inside a scaffold ($cell \cdot ml^{-1}$)
τ	Wall shear stress (Pa)
ε	porosity

centre of the scaffold. If the inlet flow rate is too high, the velocity inside the pores is consequently also too high and the seeded cells in the scaffold could be dead or detached [25].

Finally, another important point to be studied is the oxygen supply for cell culture. A low oxygen concentration could be a limiting effect on cell culture. The biological fluid contains dioxygen that flows inside the porous material. Therefore, fluid mechanics equations at the macroscale must be coupled with diffusion equations and oxygen consumption equations to ensure a whole distribution of nutrients inside the porous media. Because of the process of perfusion into a scaffold takes too much time during the in vitro experiment and due to the fact that the manipulations are complex and the process takes time and is expensive. The optimal flow rate for cell proliferation is very difficult to identify through trial-and-error.

In this paper, and in order to save time in cell culture optimization inside bioceramics, a numerical method to identify the optimal flow rate is presented. The numerical method is based on a multiscale approach and Computational Fluid Dynamics (CFD). The multiscale approach is a common approach in CFD studies because in most cases calculus stations have not enough computational capacity to solve the entire problem from a complete microscale description. Our numerical protocol is composed of six steps. Each step can be treated separately and provides key numerical physical quantities (i.e local velocities, global Darcy velocities and wall shear stress). Therefore, each step is not an integral part of a global method, but part of a stack of numerical methods that can be separately improved or treated on various calculus stations without disrupting the operating protocol. Another advantage of this protocol is that it can be adapted to various kinds of scaffolds (geometry, porosity) submitted to dynamic cell culture.

The present work focuses on dynamic cell culture into direct perfusion bioreactor . A scaffold commonly used as bone graft substitutes, with a controlled porosity, has therefore been deliberately chosen for the study [26]. This scaffold is used because of its good properties in term of cells colonization on the surface, of nutrient supply to cells and of waste evacuation. The aim of this research is to determine the optimal flow rate of a direct perfusion bioreactor in order to enhanced cell proliferation and to improve an upcoming bone reconstruction.

2. Materials and methods

In our works, we consider an entire system used to carry out dynamic cell culture. The system is so composed of:

- A DPB for which the flow rate of biological fluid can be adjusted.
- Macroporous bioceramics defined at the microscale by pores arrangement (pore size, interconnection diameter, structure) and at the macroscale by the global size and shape of the scaffold.
- A biological fluid considered as an incompressible Newtonian fluid.
- Target cells for bone substitute.

These elements are those taken into account to carry out numerical simulations.

To understand the mechanical behaviour of cell growth into a bioceramic scaffold submitted to dynamic cell culture, a Computational Fluid Dynamic (CFD) analysis on two scales is performed. A model of spherical packaging and REV was implemented using Python (Python Software Foundation. Python Language Reference, version 2.7) and its Numpy and Scipy modules [27]. At the Representative Elementary Volume (REV) scale (microscale) the mechanical stress and WSS distribution identification was performed using finite volume method (ANSYS-Fluent). ANSYS-Fluent code is used because of its advantage in memory usage and solution speed especially when working with common calculus station. Then, the statistical analysis necessary to identify the optimal range of growth stress is developed into a Python code. Finally, velocity and stress into the macroscopic bioceramic material is computed thanks to a finite element analysis with COMSOL, and more specifically the mathematics module that allows users to implement custom model. The mathematics module is so used to solve both equations based on fluid mechanics and on biology in an easier fashion than with a finite volume method. Concerning the scaffold, a classic biodegradable porous material with 100% open pores architecture is used [26].

Concerning the Wall Shear Stress at the pores scale (microscale) is calculated inside a REV. The REV is composed of a fluid part and a solid part, represented by a dozen of spherical interconnected pores. REV is built with a random distribution in order to imitate the real pore distribution of manufactured bioceramics made at the LMCPA (Laboratoire des Matériaux Céramiques et Procédés Associés, Biocetis® product of type HA40-P090825). The shear stress distribution is evaluated as a function of inlet flow rate (Q). The model used at the microscale is based on Navier–Stokes equation for an incompressible Newtonian fluid. Thanks to a previous study [28] we can give an expression of Darcy velocity from a Wall Shear Stress distribution and the evaluation of permeability into porous bioceramics with spherical interconnected pores. The published literature gives the optimal range of values of Wall Shear Stress that cells need for their growth, e.g values of WSS between 5e-3 Pa and 1e-2 Pa [29–34]. These studies are all numerical ones. Indeed, with the exception of Doppler Optical Coherence Tomography (DOCT), and even if improvements on experimental methods used to measure the wall shear stress are tangible, there are no currently available accurate experimental methods able to determine the wall shear stress on bioceramics in connection with our study case (at least to our knowledge). The DOCT was used with success on porous scaffold with a mean Wall Shear Stress between 3.8e-2 and 4.9e-2 Pa [35] but without any analysis of Wall Shear Stress on cell necrosis, so we cannot use experimental data to target the optimal range of Wall Shear Stress . μPIV methods are also in constant development and seem to be suitable for the determination of velocities. Furthermore, the correlations between experimental and numerical data are good [36]. In fact, with the μPIV

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