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Electro-osmotic pump in osteo-articular tissue engineering: A feasibility study

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Abstract. The *in vitro* construction of osteo-articular large implants combining biomaterials and cells is of great interest since these tissues have limited regeneration capability. But the development of such organoids is particularly challenging, especially in the later time of the culture, when the extracellular matrix has almost filled the initial porous network. The fluid flow needed to efficiently perfuse the sample can then not be achieved using only the hydraulic driving force. In this paper, we investigate the interest of using an electric field to promote mass transport through the scaffold at the late stage of the culture. Based on the resolution of the electrokinetics equations, this study provides an estimation of the necessary electric driving force to reach a sufficient oxygen perfusion through the sample, thus analyzing the feasibility of this concept. The possible consequences of such electric fields on cellular activities are then discussed.

Keywords: osteo-articular biomechanics; porous media; mass transport; electro-filtration; tissue engineering; bioreactor

1. Introduction

The *in vitro* construction of human bone and cartilage is of great interest since these two tissues have limited regeneration capability. The bone healing following a lesion is spontaneous in most cases and orthopedic surgery interferes only to consolidate the fracture. However, in 2.5% of the cases, nonunion can be observed six month after the injury (Phieffer and Goulet 2006). Furthermore, the impact of this type of complication raises up to 13-16% when these fractures are open and vascular-linked (Dickson *et al*. 1994). These consolidation delays need to be operated once again and they compromise quality of life and have a strong influence on the social and economic schemes. Autograft of spongy bone is still considered as the gold standard procedure for centimetric bone defect. It provides patient's osteogenic cells, a serial of osteoinductive growth factors and a structural support for the new bone formation. However, the amount of bone available for autografting is limited and associated with chronic pain and a risk of infection (Banwart *et al*. 1995). This technique is thus size limited and implies long months of recovery.

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Clinical alternatives to autograft include the use of bioceramics (e.g., calcium phosphate ceramics) or natural polymers (e.g., alginate, coral) (Oddou *et al*. 2011). Tissue engineering could then provide a powerful alternative treatment by reducing the immobilization time and addressing larger fractures. As illustrated in Fig. 1, this *in vitro* production of functional tissues requires: i) to provide an explant from the patient; ii) to isolate and amplify the specific cells; iii) to seed the adapted cells in a porous substrate which will provide a mechanical and/or chemical support for the final implant -this stage is often made in presence of biomolecules (growth factors, angiogenic molecules, chemo-attractive factors, osteo-inductive factors, etc.); iv) to cultivate the obtained organoid in an adequate bioreactor designed to ensure satisfying nutrition, oxygenation and mechanical stimulation during tissue growth; v) to finally implant the obtained engineered tissue in the patient. The quality of the final product is strongly dependant on both the seeding and culture protocols (Oddou *et al*. 2011). Nevertheless, due to the living nature of these materials, culture conditions have to be revaluated over time. Especially, when using a perfusive bioreactor for tridimensional cartilage culture, the extracellular matrix (ECM) production progressively reduces the space available for the fluid transport, making it harder to efficiently perfuse the volume of the sample. Thus, at the later time of the culture, the initial pores are almost filled with ECM, making it almost impossible to make the fluid flow through the organoid using only the hydraulic driving force. As a consequence, the cellular metabolism may be strongly modified due to the limitation of the fluid flow. This issue is currently a major limitation concerning the maximal size for the manufactured samples.

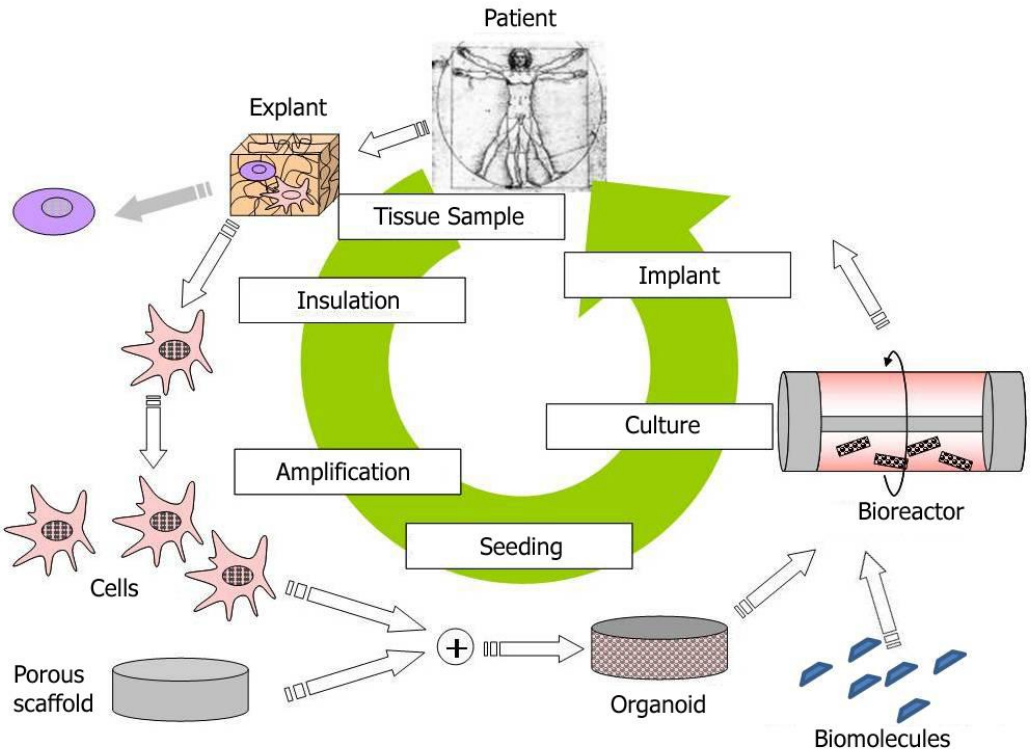

Fig. 1 Principle of tissue engineering

In this paper, we suggest to use the electrochemical properties of both the substrate and the ECM to achieve the adequate perfusion in the late stage of the tissue culture, when the pores tend to be clogged. To meet this point, another physical driving force, the electro-osmosis, is proposed. Thus, by applying electric field on the sample, the fluid perfusion through the ECM could be performed. The feasibility of this concept is analyzed thanks to an estimation of the electric field required to ensure a convenient perfusion of the organoid. A custom code initially developed to study the electro-remediation of clayey media (Lemaire *et al*. 2007) is then used to estimate the electro-osmotic flux within the ECM matrix in response to different electric field solicitations. Finally, the consequences of the order of magnitude of this electric driving force on the cellular activity and viability are discussed.

2. Method

2.1 Estimation of the adequate perfusion flow

In this study, two limit characteristic pore sizes are considered. First, the typical scaffold pore size of the biomaterials that are classically used in osteo-articular tissue engineering ranges about 10-1000 micrometers (Oddou *et al*. 2011). These large pores allow cellular seeding and fluid perfusion in the early time of the culture. They can be colonized by cells whose typical size ranges about 5-50 micrometers, and are progressively filled over time with extracellular matrix (ECM). In this study we will consider three cell types: osteoblasts, human mesenchymal stem cells (hMSCs) and chondrocytes. Furthermore, although various nutrients are implied in the success of organoids culture, we will here focus on the oxygen concentration C_{O2} of the interstitial fluid. Hyperoxic conditions (C_{O2}>0.2 mol.m^{-3}) seem to promote ECM formation - and thus a good tissue growth - for both bone (Utting *et al*. 2006) and cartilage (Grimshaw and Masson 2000). As a result, to keep good culture conditions, the perfusion flow must maintain the oxygen rate over this limit value. Consequently, the limit perfusing velocity V ensuring the adequate culture conditions can be roughly estimated by

$$V = \frac{nQL}{C_{O2}} \tag{1}$$

where n represents the cellular density, Q the cellular consummation rate of oxygen and L the sample thickness. As given in Table 1, Q depends on the cell type, giving two limit perfusion

Table 1 Physiological data used to estimate the perfusion velocities ensuring a sufficient oxygen rate

Parameter	Value	Reference
$\boldsymbol{n}$	6.2×10^{12} cell.m^{-3}	Porter *et al.* (2009)
$\boldsymbol{L}$	10^{-2} m	Oddou *et al.* (2011)
$\boldsymbol{Q_H}$	4×10^{-17} mol.cell^{-1}.s^{-1}	zur Nieden *et al.* (2007)
$\boldsymbol{Q_L}$	4×10^{-18} mol.cell^{-1}.s^{-1}	Malda *et al.* (2004)
$\boldsymbol{V_H}$	1.24×10^{-5} m.s^{-1}	Eq. (1)
$\boldsymbol{V_L}$	1.24×10^{-6} m.s^{-1}	Eq. (1)

velocities. If the cellular consummation rates of oxygen for bone cells (Komarova *et al*. 2000) and for stem cells (zur Nieden *et al*. 2007) are comparable and noted Q_H, the one for the chondrocytes Q_L is one order of magnitude lower (Malda *et al*. 2004). The resulting high and low estimations of the perfusion velocities are similarly indexed V_H and V_L.

2.2 Corresponding driving pressure gradients

According to the classical Darcy law, the hydraulic gradients G_H corresponding to these required velocities are expressed by

$$G_H = -\frac{\mu_f V}{K} \tag{2}$$

where $\mu_f = 1\times10^{-3}$ Pa.s is the fluid dynamic viscosity (water at room temperature) and K the intrinsic permeability of the medium. Since the values of the intrinsic permeability of cartilagineous and lacuno-canalicular tissues are similarly very low, being about 10^{-18} m^2 (Reynaud and Quinn 2006, Lemaire *et al*. 2012), the hydraulic driving pressure gradients that would be necessary to ensure the suitable perfusion velocities shown in Table 1 should be about 1 to 10 GPa.m^{-1}. Besides the technical problems inherent to such a high pressure force, these values are prohibitively high for the cells causing irremediable damages in the new tissue (Buehler and Yung 2009).

Thus, classical perfusion pump are not able to provide a sufficient flow, the downstream part of the cultivated tissue being out of reach. It is so necessary to propose another physical driving effect to ensure the fluid flow within the organoid. Since the ECM is mainly composed of glycan chains that present a negative charge, we propose to use an electro-osmotic pump to force the fluid flow.

2.3 Principle of electro-osmosis

Since the ECM can be seen as a charged porous medium with nanometric pores, the electro-kinetics phenomena characterizing the mass transport through other electrically charged porous media such as clay barriers could be adapted. Electro-osmosis is indeed a classical technique in the domain of soil electro-remediation (Acar *et al*. 1995, Lemaire *et al*. 2007). Its principle consists in generating a hydraulic flux in response to an electric field from the anode toward the cathode. This electro-viscous phenomenon is associated with the electro-migration of the charged species, the cations moving toward the cathode and the anions toward the anode. The nanometric explanation of these coupled phenomena consists in the negative charge characterizing the pores surface. This charge is compensated by the development an electrical double layer due to the adsorption of cations on the surface (Stern layer) and the formation of an outer diffuse layer in the fluid composed of mobile cations (Hunter 1981, Lemaire *et al*. 2011b). When submitted to a macroscopic electric field, the mobile charge population of the double-layer moves under the electro-migrative effect. Due to the viscous drag interaction, the ions pull the liquid with them resulting in an electro-osmotic seepage flow.

Based on our experience of these multiphysics phenomena in the framework of clayey media (Lemaire *et al*. 2007, Lemaire *et al*. 2010a) or bone *in vivo* behaviour (Lemaire *et al*. 2008, Lemaire *et al*. 2010b, Lemaire *et al*. 2013), we propose to quantify the required electric field to obtain a convenient perfusion velocity. Since the pore size δ and the pore surface electric property

(here the surface charge σ) are sensitive elements in the determination of the electro-osmotic flux (Sansalone *et al*. 2013), different values of these parameters have been considered.

3. Results and discussion

3.1 Numerical estimation of the electro-osmotic velocity

As shown by our earlier studies on multiphysics effects in cortical tissue, due to the double-layer effects, the bone fluid flow in the very thin pores (diameter of a few nanometers) requires to take into account the electro-chemical effects additionally to the sole hydraulic effect (Lemaire *et al*. 2006). More precisely, it has been shown that, if chemo-osmotic can be neglected, electro-osmosis may become the main driving effect for nanometric pore sizes, when the double layers tend to occupy the entire pore volume (Lemaire *et al*. 2012).

Thus, neglecting osmosis, the coupled Poiseuille law that represents the average fluid velocity vector v developing in the nanopores reads (Lemaire *et al*. 2011a)

$$v = -K_P\, G_H - K_E\, G_E \tag{3}$$

Here, in addition to the hydraulic part of the flow induced by the hydraulic gradient vector G_H and quantified by the Poiseuille hydraulic conductivity K_P, there is an electro-osmotic flow in response to the electric driving gradient vector G_E and quantified by the electro-osmotic permeability K_E. The expressions of these two transport properties are explicitly given for pores mimicking the clayey platelets (Lemaire *et al*. 2007) and for cylindrical and annular pores mimicking the bone canaliculi (Lemaire *et al*. 2006, Lemaire *et al*. 2008). If the Poiseuille parameter only depends on the pore geometry and the fluid viscosity, the electro-osmotic one also depends on the fluid permittivity $\varepsilon=\varepsilon_r\,\varepsilon_0$ (ε_r being the relative permittivity and ε_0 the vacuum permittivity), the temperature T, the salinity c and the reduced double layer potential $\bar{\varphi}$. This electric potential follows the Poisson-Boltzmann equation

$$\Delta\bar{\varphi} = \frac{1}{L_D{}^2}\sinh\bar{\varphi} \tag{4}$$

where the Debye length L_D roughly characterizes the thickness of the electrical double layer. Note that this is this quantity that contains the influence of the fluid permittivity ε, the temperature T and the ionic salinity c according to

$$L_D = \sqrt{\frac{\varepsilon RT}{2F^2 c}} \tag{5}$$

where F and R are the Faraday and the gas constants, respectively. Using the negative surface charge of the pores σ and the normal vector of this surface $\mathbf{n}$, the associated boundary condition is

$$\nabla\bar{\varphi}.\mathbf{n} = \frac{F\sigma}{RT\varepsilon} \tag{6}$$

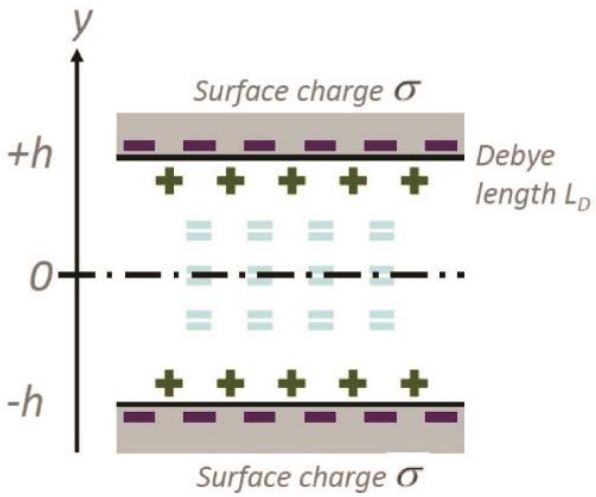

Fig. 2 Sketch of the elementary Cartesian pore presenting a negative surface charge

Due to the highly non-linear nature of this equation, a custom code was developed to solve it (Lemaire *et al*. 2007, Lemaire *et al*. 2013) and thus we obtain the electro-osmotic conductivity from the double layer potential. This code is based on the recursive resolution of the Cartesian Poisson-Boltzmann problem proposed by Derjaguin *et al*. (1987). Thus, a model of straight channel (half-size $h \equiv \delta/2$) is built for a representative pore filled with a water-saturated electrolyte (salinity c) and presenting a negative surface charge density σ (see Fig. 2).

3.2 Required electric driving forces

Since the electro-osmotic process is only viewed as a supplementary driving force in the late stage of the tissue culture when the hydraulic gradient would be insufficient to ensure a convenient perfusion, the electric fields needed to reach the adequate perfusion flow level are calculated from Eq. (3) for pore sizes ranging between 2 and 40 nm. As it is useless for such thin pores, the hydraulic gradient is set to zero. Moreover, the surface charge density being unknown, we choose to investigate physiologically reasonable values between -0.1 and -0.4 $C.m^{-2}$. This order of

Table 2 Physiological data used to estimate the perfusion velocities ensuring a sufficient oxygen rate

Parameter	Value	Reference
$\boldsymbol{L}$	10^{-2} m	Typical biomaterial size
$\boldsymbol{T}$	310 K	Human body temperature
ε_r	80	Salt water relative permittivity
μ_f	0.6×10^{-3} Pa.s	Salt water viscosity at the human body temperature
σ	{-0.1 to -0.4} $C.m^{-2}$	Mattern *et al.* (2008), Harding *et al.* (2005)
$\boldsymbol{c}$	10^{-2} M	Lemaire *et al.* (2008)
$\boldsymbol{h}$	$\{1 \text{ to } 20\}\times10^{-9}$ m	-

magnitude of the the surface charge has been obtained considering either the glyco-amino-glycan surface charge (Mattern *et al*. 2008) or the cationic absorption capacity on the hydroxyapatite surface (Harding *et al*. 2005). The other parameters involved in the determination of the electric fields are provided in Table 2.

In Fig. 3, we present the required electric fields which are necessary to ensure the convenient perfusion velocity. The estimations corresponding to different cell types are investigated: on the top (see Fig. 3(a)), the high flux estimations corresponding to bone and stem cells, and on the bottom (see Fig. 3(b)), the low flux estimations corresponding to chondrocytes.

Let us first discuss the influence of the pore size. In either case, the value of the driving electric field stabilizes for pore half sizes greater than 10 nm. This limit corresponds to the typical double layer thickness. Thus, above this limit pore size, the volume of the fluid occupied by mobile counterions remains almost constant, causing this continuous electro-viscous phenomenon. However, for nanometric pores, the required electric driving force may change significantly. The thinner the pores are, the more important the required electric driving force becomes.

Let us now focus on the surface charge of the pores. Its influence on the electro-osmotic efficiency is clearly visible: for a given pore size, the higher the surface charge, the lower the required driving force. Note that there is a tendency for this charge-dependency to soften for high surface charges.

To assess the feasibility of our electro-osmotic pump, possible side-effects have to be analyzed. Indeed, electric fields may have different consequences on proliferation, viability, mineralization activity, cellular migration.

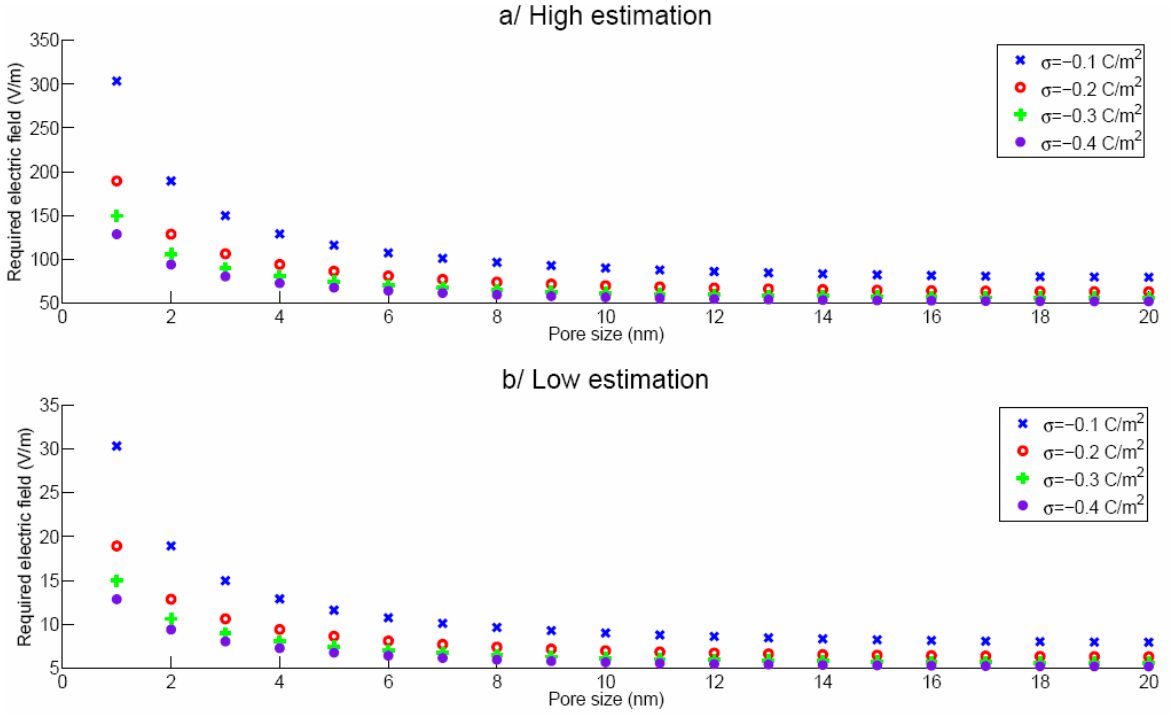

Fig. 3 Required electric driving forces: (a) high estimations corresponding to bone and stem cells; (b) low estimations corresponding to chondrocytes

3.2.1 Side-effects on bone or stem cells

Comparing the values obtained in Fig. 3(a) with those found in the literature, it is first to notice that the needed electric driving force stays beyond the electric stimulations used to promote proliferation and/or extra-cellular matrix production for osteoblast-like cells (Hartig *et al*. 2000, Meng *et al*. 2011). Indeed, the electric fields used for these types of cellular stimulation are above 200 $V.m^{-1}$ whereas the required electric fields to ensure a suitable perfusion stay below 100 $V.m^{-1}$. Furthermore, according to Curtze *et al*. (2004), for electric fields of this magnitude, there is no impact on osteoblast-like cells *via*bility, and therefore no duration limit for the electro-stimulation.

Similarly, cellular reorientation and migration do not seem to be at risk either (Sun *et al*. 2006, Hammerick *et al*. 2010). However, a possible electrically-induced differentiation is possible for such values of the electric field (Yamada *et al*. 2007).

3.2.2 Side-effects on chondrocytes

Concerning chondrocytes, even if the needed electric fields seem to be beyond the lethal values presented in Protsenko *et al*. (2011), it matches a range where proliferation is inhibited (Armstrong *et al*. 1988), which would be a major obstacle for tissue development. To limit this unfavourable effect, it would be necessary to use lower driving electric forces by, for instance, increasing the oxygen concentration. Note that the control of other biochemical parameters, such as the pH, may be interesting to study under hypoxia conditions (Das *et al*. 2010).

3.3 Limitations of the present approach

The efficiency of the model-driven design of an experimental device is highly determined by the relevance of the model assumptions and the accuracy of its parameters.

Here, for the sake of simplicity, we considered a Newtonian fluid to perfuse the scaffold. Moreover, if the perfusing flow occurs in reality within a 3D-structure, our feasibility study involved a 1D-model. This assumption can be convenient due to two arguments. On the one hand, the nanometric pore size implies very low Reynolds numbers and thus 3D effects such as possible recirculation remain very limited. On the other hand, the Poisson-Boltzmann equation that governs electrostatics in the nanopore volume can not be linearized according to the Debye-Hueckel approximation for such thin pores. To the authors knowledge, a reliable approximation of this equation in a 3D context does not exist. As a consequence, we preferred to adopt a 1D resolution.

Finally, interested readers are invited to consult the parameters sensitivity analysis of the model proposed in a previous work (see Sansalone *et al*. 2013).

4. Conclusions

This study proposed a model-driven approach to simply investigate the feasibility of using an electro-osmotic pump to ensure a suitable perfusion in a very low porous cultured tissue. This typically corresponds to the final stage of a perfusive 3D tissue culture in a bioreactor when the extracellular matrix tends to clog the pores. Note that new biporous scaffolds have been recently developed for tissue engineering applications (Le Droumaguet *et al*. 2014). The macropores allow cells to proliferate while the nanopores are useful for the cellular adhesion and, using the electro-osmotic process, the passage of nutrients and cellular waste products.

The strategy was here to check whether the perfusion velocity was large enough to ensure good

perfusion conditions. Two cases corresponding to different types of cells have been studied. Through this study, even if the cellular viability does not seem to be strongly affected when compared to observations described in the literature, we showed that the more critical situation corresponds to the culture of chondrocytes since the cellular proliferation may be modified by electric fields typically corresponding to the required electric driving force. To overcome this problem, the influence of parameters such as oxygen concentration and pH should be experimentally studied.

Acknowledgments

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