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# Elaboration and evaluation of alginate foam scaffolds for soft tissue engineering

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ABSTRACT

Controlling microarchitecture in polymer scaffolds is a priority in material design for soft tissue applications. This paper reports for the first time the elaboration of alginate foam-based scaffolds for mesenchymal stem cell (MSC) delivery and a comparative study of various surfactants on the final device performance. The use of surfactants permitted to obtain highly interconnected porous scaffolds with tunable pore size on surface and in cross-section. Their mechanical properties in compression appeared to be adapted to soft tissue engineering. Scaffold structures could sustain MSC proliferation over 14 days. Paracrine activity of scaffold-seeded MSCs varied with the scaffold structure and growth factors release was globally improved in comparison with control alginate scaffolds. Our results provide evidence that exploiting different surfactant types for alginate foam preparation could be an original method to obtain biocompatible scaffolds with tunable architecture for soft tissue engineering.



## 1. Introduction

For the past decades, there has been a growing interest in the use of Mesenchymal Stem Cells (MSCs) to regenerate biological tissues after several acute and chronic diseases. After an initial focus on their capacity to differentiate into mesodermal lineage, they are now acknowledged for their positive effects attributed to their paracrine activities, which allow direct regeneration as well as indirect modulatory effects on damaged and diseased tissues. MSCs secrete paracrine factors which promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and decrease inflammatory/immune reactions (Caplan, 2007; Li and Ikehara, 2013; Souidi et al., 2013). Such therapeutic properties are particularly effective in ischemic diseases treatment of the heart (Léobon et al., 2009; Panfilov et al., 2013), kidneys (Alfarano et al., 2012; Furuichi et al., 2012) and lungs (Chen et al., 2012; Yip et al., 2013). In these treatments, MSCs are delivered to the targeted organ by injection into the perfusing artery or directly into the tissue surrounding the damaged area. Unfortunately, benefits of such therapeutic approaches are limited by poor cell retention and early cell death at the injury site after implantation. Indeed, several studies have reported that more than 80–90% of transplanted cells die within the first 72 h after injection (Maurel et al., 2005; Toma et al., 2002). Multiple mechanisms are involved in these early cell losses including hypoxia, local inflammation and mechanical stress occurring during cell administration. Improvement of cell concentration and viability at the injury site, in order to promote their therapeutic activity, is becoming a priority in the field of cell therapy.

One promising strategy is to associate MSCs with a biocompatible material that protects and concentrates them on the damaged area. The ideal scaffold should improve viability of grafted MSCs, preserve their paracrine activity and provide an artificial matrix

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allowing medium/long term cell survival as well as their secretion function. In addition, the mechanical properties of the selected material must not only be compatible with soft tissues but also appropriate for surgery manipulations during implantation on the damaged tissue. Scaffold architecture is another critical parameter that could affect the biological activity of entrapped cells and the fate of the implanted device. More specifically, it has been reported that pore size distribution and pore interconnectivity affect cell morphogenesis (Zmora et al., 2002), stem cell behavior and implant's colonization by host cells (Salem et al., 2002; Souidi et al, 2013; Toma et al., 2002; Zeltinger et al., 2001).

Among materials used for cell therapy, natural polymers seem to be particularly adapted in terms of biocompatibility (Lee and Mooney, 2001). In that regard, alginates are among the most widely used polymers (Andersen et al., 2015; Bidarra et al., 2014; Giovagnoli et al., 2015; Ruvinov and Cohen, 2016; Silva et al., 2015 ) due to their low toxicity after purification, gelling properties (under conditions compatible with biological activities: 37 °C, pH 7.4 . . . ), structural resemblance to the extracellular matrix (considered to be at the origin of their excellent biocompatibility), and relatively low cost. Regarding their origin and chemical structure, alginates are naturally occurring anionic linear (unbranched) polysaccharides, which can be extracted from kelp, brown seaweed and some bacteria. They are salts of alginic acid consisting of 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) residues organized in regions of sequential G units (G-blocks), regions of sequential M units (M-blocks) and regions of G and M units atactically organized. Their sol–gel transition properties are based on the formation of a stiff "egg-box" structure due to divalent cations selective binding to the G-blocks of two adjacent polymeric chains (Grant et al., 1973). The major issue limiting the widespread use of alginate hydrogels as tissue engineering scaffolds is the possible exchange of divalent cations with monovalent cations over time (Bajpai and Sharma, 2004), resulting in crosslinks dissociations in the gel's network followed by a mechanical degradation. However, alginates' mechanical behavior is easily modifiable by different crosslinking or by changing the type and/or the molecular weight distribution to match the required stiffness of host tissues (Augst et al., 2006). Moreover, the degradation rate depends not only on alginates' characteristics, but also on the device's dimensions and implantation site. For example, alginate microspheres injected under the renal capsule were almost intact 4 weeks after implantation (Trouche et al., 2010); it was also the case for G-type alginate scaffolds implanted on rat myocardium but not for M-type alginate scaffolds (Ceccaldi et al., 2012). Thus, an accurate choice of alginate type/properties could allow a wide range of biomedical applications.

The biocompatibility of alginates has been extensively described in the literature and for the last few decades, the scientific community has worked to established efficient methods to produce alginates with high purification grades and limited amount of polyphenols, endotoxins and protein residues which can impact the inflammatory reaction after implantation (Klock et al., 1997; Leinfelder et al., 2003; Tam et al., 2006). In general, alginates are not known to be biologically active. In fact, protein adsorption and cell attachment are low due to their high water content, dense negative surface charge, and the lack of molecular recognition by cell surface receptors (Dvir-Ginzberg et al., 2008; Gandhi et al., 2013; Glicklis et al., 2000). This particularity of alginates, combined with their strictly local effect (on the application site), have allowed the material to be qualified as safe for human application. Furthermore, several clinical trials using alginate-based medical devices are currently in progress (AUGMENT-HF: NCT01311791; PRESERVATION 1: NCT01226563; NCT01734733; NCT00521937) or completed (GLP-1 CellBeads®: NCT01298830; DIABECELL®: NCT00940173; NCT01396304), demonstrating the growing interest in the use of this polymer for biomedical applications.

Regarding tissue engineering applications, macroporous three-dimensional (3D) alginate scaffolds are of particular interest. Indeed, compared to non-macroporous hydrogels they provide to cells a biomimetic environment, allow improved cell infiltration, better diffusion of solutes, nutrients and oxygen, as well as enhanced waste removal (Shapiro and Cohen, 1997). Additionally, despite the non-adhesive nature of alginate polymers, cells are efficiently incorporated and retained within 3D alginate sponges due to the porous structure of the matrix whereas they are not on bi-dimensional (2D) alginate films (Dvir-Ginzberg et al., 2008; Glicklis et al., 2000). A number of studies have shown benefits when using alginate macroporous scaffolds for 3D cell culture (Sapir et al., 2011; Caplan, 2007; Li and Ikehara, 2013; Shachar and Cohen, 2003; Shapiro and Cohen, 1997; Zieber et al., 2014) and for soft tissues regeneration (Dvir et al., 2009; Dvir-Ginzberg et al., 2008; Leor et al., 2000). In particular, foaming alginates has allowed obtaining highly porous scaffolds with tunable morphology and mechanical characteristics according to the type and concentration of alginate used as well as the source of gelling ions (Andersen et al., 2012, 2014a). In addition, alginate foams appeared to be highly compatible for cell entrapment, prolonged 3D cell culture and retrieval of NHIK 3025 and NIH: 3T3 cells (Andersen et al., 2014b). In our study, we wished to produce foam-based alginate porous scaffolds specifically adapted for MSC use in cell therapy, *i.e.* tailored for MSC immobilization and improvement of their secretion ability. For alginate foaming, we have chosen to use surfactants coming from the polysorbates (Montanox®) and the poloxamers (Pluronic®) families, as they are non-ionic, water soluble (hydrophilic–lipophilic balance >8), biocompatible, and certified for biomedical applications (Andersen et al., 2012; Bueno et al., 2014; Eiselt et al., 2000; Fowler et al., 2002; Inzana et al., 2014; Tadros, 2005; Vashi et al., 2008). More precisely, we used four of these surfactants as we had observed them to be compatible with MSC culture (based on a preliminary evaluation of their cytotoxicity and water solubility): Montanox 20, Montanox 80, Pluronic 127 and Pluronic 108. Mixing each one of them with an alginate solution followed by a freeze-drying, permitted the generation of four different foam-based scaffolds. They were characterized with regard to their architecture, porosity, mechanical properties and cell-seeding ability with functional MSCs. Finally, cell viability as well as cell secretion function were also investigated in order to ascertain the most promising formulations for soft tissue cell therapy.

## 2. Materials and methods

Ultrapure MVG sodium alginate with a M/G ratio of 0.47 (determined by $^{1}H$ NMR measurement) was purchased from Provona Biopolymer Inc. (Novamatrix, Norway). Sodium bicarbonate was furnished by Cooper (France). Montanox and Pluronic surfactants were provided by Seppic (France) and BASF Corporation (France), respectively. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) sodium salt was purchased form Sigma–Aldrich, France. Sodium chloride (NaCl) and calcium chloride dehydrate ($CaCl_2{\cdot}2H_2O$) were purchased from VWR. Reagents used for *in vitro* cell culture were α-Minimum Essential Medium (α-MEM, Invitrogen, San Diego, CA, USA) supplemented with 10% fetal calf serum (Hyclone, Logan, UT, USA) and ciprofloxacin (10 mg $ml^{-1}$; Bayer Schering Pharma, Germany).

### 2.1. Macroporous scaffolds elaboration

Solutions of 3% (w/w) MVG alginate were prepared in iso-osmotic saline solution during 30 min at 1800–2000 rpm