Materials Science and Engineering C 36 (2014) 77–83

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Process engineering of high voltage alginate encapsulation of mesenchymal stem cells

Oleksandr Gryshkov [a,*], Denys Pogozhykh [a], Holger Zernetsch [a], Nicola Hofmann [a], Thomas Mueller [b], Birgit Glasmacher [a]

[a] Institute for Multiphase Processes, Leibniz University Hannover, D-30167 Hannover, Germany
[b] Institute for Transfusion Medicine, Medical School Hannover, D-30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form 12 November 2013
Accepted 28 November 2013
Available online 7 December 2013

Keywords:
Alginate encapsulation
High voltage
Electro-spraying
Spherical beads
Scaling up
Mesenchymal stem cells

ABSTRACT

Encapsulation of stem cells in alginate beads is promising as a sophisticated drug delivery system in treatment of a wide range of acute and chronic diseases. However, common use of air flow encapsulation of cells in alginate beads fails to produce beads with narrow size distribution, intact spherical structure and controllable sizes that can be scaled up. Here we show that high voltage encapsulation (≥15 kV) can be used to reproducibly generate spherical alginate beads (200–400 μm) with narrow size distribution (±5–7%) in a controlled manner under optimized process parameters. Flow rate of alginate solution ranged from 0.5 to 10 ml/h allowed producing alginate beads with a size of 320 and 350 μm respectively, suggesting that this approach can be scaled up. Moreover, we found that applied voltages (15–25 kV) did not alter the viability and proliferation of encapsulated mesenchymal stem cells post-encapsulation and cryopreservation as compared to air flow. We are the first who employed a comparative analysis of electro-spraying and air flow encapsulation to study the effect of high voltage on alginate encapsulated cells. This report provides background in application of high voltage to encapsulate living cells for further medical purposes. Long-term comparison and work on alginate–cell interaction within these structures will be forthcoming.

1. Introduction

Semi-permeable membranes (SPM) made of alginate have been widely used to entrap living cells and cellular therapeutics for cell-based therapies [1,2]. It protects living cells from the response of the immune system of the host and provides 3D arrangements for cells by mimicking the extra cellular matrix [3,4]. Degradation rate of alginate beads and their biocompatibility can be previously matched to improve release of therapeutics and enhance long-term performance of such a therapy [5].

Alginate is known to be a linear block co-polymer containing (1,4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) and being extracted from different types of algae as well as bacteria. Alginate forms a gel due to cross-linkage with divalent cations, such as Ca^{2+} and Ba^{2+}. Mechanical properties of resulting alginate beads depend on: 1) source of alginate, 2) type of cross-linking and 3) cross-linking density [6]. A lot of efforts have been made to stabilize the alginate structure via internal and covalent cross-linking [7,8] and allow attachment of mammalian cell types by incorporating RGD peptides [9]. Attention has also been paid on application of poly-cationic coatings of alginate beads, such as poly-L-ornithine and poly-L-lysine [10]. Interestingly, such coatings allowed generating of alginate beads with solid or liquid core (beads and capsules respectively) and were also shown to stabilize the alginate structure and improve survival of encapsulated cells after cryopreservation for long-term storage [11]. Despite this, above-mentioned coated alginate beads possessed an increased inflammation response in-vivo as compared to uncoated barium and calcium alginate beads [12].

There is a range of methods allowing encapsulation of living cells in alginate beads, such as air flow, electro-spraying, micro-nozzle array and ink-jet [13–16]. Nowadays, air flow and electro-spraying methods have already found their industrial applications and being produced as ready-to-use commercial devices (NovaMatrix/FMC BioPolymer, Switzerland, BUCHI Labortechnik AG, Switzerland and NISCO Engineering AG, Switzerland). These manufacturers also support their equipment with Good Manufacturing Practice (GMP) compliant documentation, which is necessary for further clinical application. Despite this, the commercially available encapsulation devices are expensive and are not always suitable to generate polymer beads containing living cells, especially stem cells, under sterile

Abbreviations: MSCs, mesenchymal stem cells; RGD, arginylglycylaspartic acid; ES, electro-spraying; AF, air-flow; WS, washing solution; SPM, semi-permeable membrane.

* Corresponding author. Tel.: +49 511 7623827; fax: +49 511 7623031.
E-mail addresses: gryshkov@imp.uni-hannover.de (O. Gryshkov), pogozhykh@imp.uni-hannover.de (D. Pogozhykh), zernetsch@imp.uni-hannover.de (H. Zernetsch), hofmann@imp.uni-hannover.de (N. Hofmann), mueller.thomas@mh-hannover.de (T. Mueller), glasmacher@imp.uni-hannover.de (B. Glasmacher).

0928-4931/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.msec.2013.11.048

conditions with narrow size distribution and intact structure [14]. Although small-sized alginate beads have currently been produced, the large-scale expansion of such methods for clinical application is still a challenge [15]. As cellular material we utilized primary cultures of mesenchymal stem cells (MSCs) from the bone marrow from a small non-human primate, the common marmoset (*Callithix jacchus*) from the northeast of Brazil. The animal is readily used for biomedical research due to its close genetic proximity to the human and its stem cells have been intensively utilized for culture, differentiation and reprogramming approaches [17,18].

At first, we aimed to evaluate whether high voltages are able to generate alginate beads with narrow size distribution while avoiding ruptured structure. Afterwards we analyzed the effect of high voltages on viability and proliferation of mesenchymal stem cells post-encapsulation and after cryopreservation.

2. Materials and methods

2.1. Alginate: source and sterilization

Medium viscosity alginate sodium salt from brown algae was purchased from Sigma Aldrich (A2033, M_w = 80–120 kDa, viscosity ≈ 2000 cP (2%, 25 °C)) and dissolved in 10 mM HEPES buffered saline (pH = 7.4) to a required concentration by gentle shaking overnight. Aliquots of alginate solution were kept at 4 °C until use. Prior to experiments (optimization of process parameters and cell encapsulation) alginate solution was sterilized using 0.8 μm, 0.45 μm (Carl Roth GmbH, Germany) and 0.2 μm (TPP, Biochrom, Germany) filter sets. Membrane filtration of alginate solution does not cause significant changes in molecular weight and viscosity of alginate [19]. Sterile alginate solution was transferred to 10 ml sterile syringes and used for further purposes.

2.2. Encapsulation methods

2.2.1. High voltage electro-spraying

The high-voltage electro-spraying encapsulation (ES) applies voltage higher than 15 kV for detaching alginate beads from the nozzle [20]. Here, the alginate solution is kept in a syringe and pumped through the nozzle (27G, 0.4 mm, BBraun, Melsungen, Germany) at defined flow rate. Alginate gel is detached from the tip of the needle by applying high voltage between the positive and grounded electrode. Next, the alginate solution is transported to the bath containing stirred cross-linking solution with Ca^{2+} ions (Fig. 1A, B). In the following calcium ions replace sodium ions, causing a shrinking of alginate during the short period of time, thereby forming a gel-like structure (Fig. 1B1–B3).

2.2.2. Air flow

As a comparative approach we used air flow encapsulation (AF) to study the effect of high voltage on viability and proliferation of encapsulated MSCs. Such method is the most commonly used technique to encapsulate living cells in alginate. The general description of this method can be found elsewhere [13]. In brief, this method uses a high velocity air current to overcome the surface tension of the alginate solution resulting in bead detachment. Simple AF equipment has been established in our lab and it was used as a comparative approach to high voltage. Process parameters of AF (flow rate of alginate, distance from the co-axial nozzle and gelling bath, air flow velocity) have been previously optimized to generate alginate beads with narrow size distribution [21]. The process parameters (alginate flow rate 10 ml/h, spraying distance 10 cm, air flow 150 l/h) were kept constant to produce beads comparable in diameter to the electro-sprayed ones (300 μm).

2.3. Optimization of process parameters

Alginate concentration (1–3%, w/v), alginate flow rate (0.5–10 ml/h), applied voltage (10–30 kV), concentration of cross-linking ions (20–500 mM) and spraying distance (5–22.5 cm) were taken as process parameters for optimization studies. Each process parameter was optimized respectively, while the other ones were kept constant: alginate concentration 1.5% (w/v), flow rate 2 ml/h, cross-linking solution 100 mM, and applied voltage 20 kV (in case of spraying distance optimization the applied voltage was kept at 23 kV). The beads were incubated to cross-link for 10 min and washed twice in a 15 ml falcon tube with washing solution (WS) of 10 mM HEPES (Carl ROTH GmbH, Germany) buffered saline containing 1.5 mM $CaCl_2$ (Fluka Analytical, Sigma Aldrich, Germany, pH = 7.4). Beads were analyzed using Carl Zeiss Axiovert microscope in bright field while cross-linking calcium ions in WS prevented alginate beads from swelling [22]. Approximately 100 beads were randomly imaged and analyzed using AxioVision software (Rel. 4.7, Carl Zeiss, Germany).

2.4. Cell culture and encapsulation

MSCs derived from the Common marmoset monkey *C. jacchus* were cultured in DMEM containing 15% fetal bovine serum (FBS, Biochrom, Germany), 1% penicillin/streptomycin and 1% ascorbic acid in 10 cm tissue culture dishes (TPP, Biochrom, Germany) in a humidified incubator at 37 °C and 5% CO_2. Cells were harvested at passage 9 using Trypsin/EDTA solution followed by centrifugation at 200 *g* for 5 min. Membrane integrity of MSCs prior to encapsulation was assessed by Trypan Blue exclusion method using ViCell XR counter

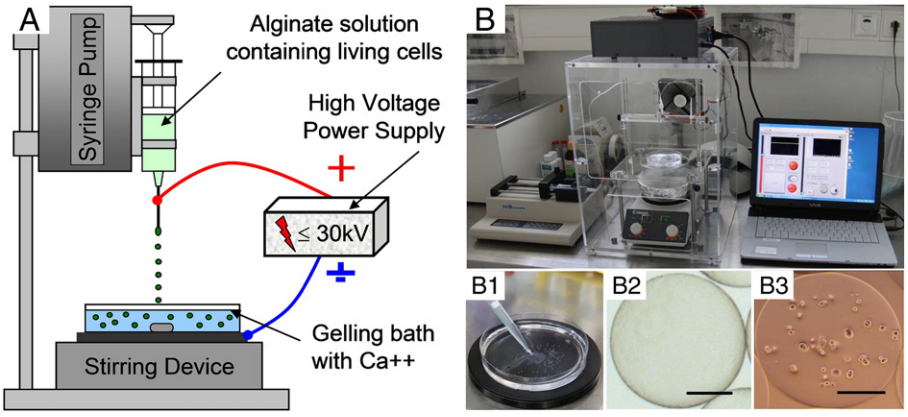

Fig. 1. Engineering of high-voltage encapsulation of MSCs in alginate beads. (A) Schematic representation of high voltage electro-spraying. (B) Electro-spraying equipment. Alginate immediately turns to a gel (B1) thus forming alginate beads with (B3, $1 * 10^6$cells/ml) or without entrapped living cells (B2). (Scale bars 100 μm).