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Expansion properties of alginate beads as cell carrier in the fluidized bed bioartificial liver

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ABSTRACT

Liquid fluidization offers the advantage of homogeneous and controllable expansion of suspended particles with limited pressure drop and favorable heat and mass transfer conditions. Bioreactors can exploit such potential and fluidized-bed-based biomedical devices acting as bioartificial liver have proved an effective alternative to other solutions. In such a systems, alginate beads hosting individual or agglomerated hepatic cells are fluidized by a perfusion medium, with the bioreactor performance significantly affected by the hydrodynamics and mass transfer. In the present work, the intrinsic and fluidization properties of alginate beads are carefully analyzed using two rigs at different scales. Appropriate alginate beads were prepared by the technique of alginate drop gelation in calcium chloride. The beads were characterized in terms of size distribution and density, obtaining a Sauter mean diameter $D = 813$ μm and density $\rho = 1020$ kg/m^3. The latter value is very close to usual perfusion fluids, requiring careful evaluation also of the liquid properties. Expansion properties were evaluated for free alginate beads (i.e. without hepatic cells) using appropriate saline solutions as fluidization medium. Bed expansion tests over a wide range of voidage values have been conducted in a 1-cm diameter column used for perfusion during in vitro experiments as well as in a 10-cm diameter column close to human size bioreactor at ambient (20 °C) conditions. Expansion data, terminal velocity measurements and velocity-voidage plots are reported and elaborated on in terms of Richardson-Zaki parameters.

1. Introduction

The homogeneous expansion behavior of liquid-fluidized beds is exploited in various fields such as minerals engineering and biotechnology [1,2]. Innovative fluidized bed bioreactor concepts have been also explored for applications as bioartificial organs, particularly the bioartificial liver [3]. Due to the high cost and shortage of organ donors, liver transplantation suffers from severe limitations. Liver tissue constructs consisting of functional cells and artificial materials are being greatly studied for their applications in the field for organ replacement and for in vitro studies on drug development and metabolic diseases [4]. The concept of a two-phase bioreactor with a fluidized bed of alginate beads containing immobilized hepatocytes was initially proposed by Doré and Legallais [5,6] although similar devices could be found in earlier patents [7].

The use of alginate beads in which hepatocytes are entrapped seems very promising because this spherical configuration offers, in addition to the hepatocyte anchorage, the largest surface area to volume ratio for optimal solute and oxygen transfer between the hepatocytes and the perfusion fluid (either blood or plasma) in both directions [8].

Application of the fluidized bed bioartificial liver requires a conceptually simple loop [8] (Fig. 1). The patient's blood is withdrawn and separated into plasma and blood cells. Plasma treatment is carried out by perfusion through a fluidization column hosting the hepatocytes. Plasma and blood cells are eventually mixed again and returned to the patient.

Hepatic cells in the form of spheroids immobilized within alginate beads in fluidized bed devices have been recently subject to tests and successfully validated in preclinical trials with respect to biological functions and metabolic activity [9]. The effect of alginate preparation and beads size on the mass transfer and metabolic activity has been investigated by Gautier et al. [8] and other groups [10]. Generally, it has been shown that the overall performance of the fluidized bed bioartificial liver strongly relies on the effective hydrodynamics and mass transfer in the bioreactor. Unfortunately, in real applications the expansion of the beads is not clearly visible even in transparent bioreactors, as the fluidization medium may be opaque. As it is well known, measures of the pressure drop are also of little use to characterize expansion once the minimum fluidization velocity is overcome. Therefore, the ability

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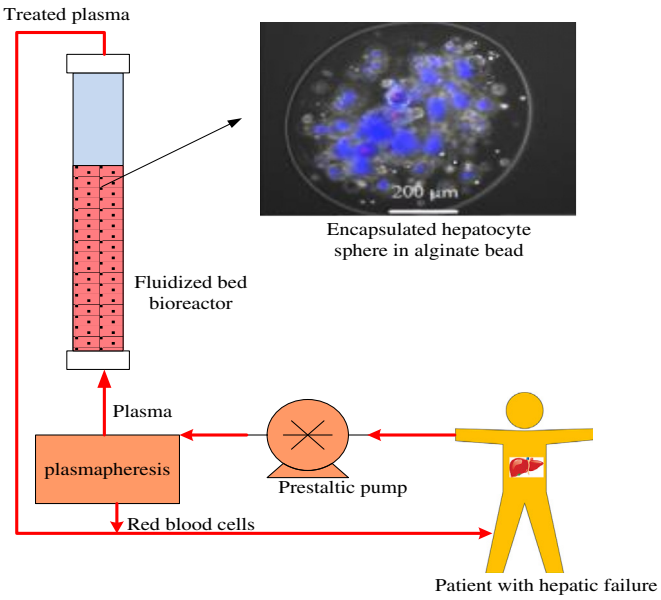

Fig. 1. Principle of the fluidised bed bioartificial liver.

to accurately predict the expansion properties of the beads' bed is very important to the efficient use of the bioreactor.

The main objective of this work is to provide data and analysis useful to the selection of the optimal hydrodynamic regime in the design and scale-up of bioartificial devices based on the fluidized bed of alginate beads. This is achieved first by a careful evaluation of the properties of alginate beads with specific respect to the characteristics influencing fluidization, including density (pure alginate and/or presence of hepatocytes), size distribution, swelling characteristics. Fluidization properties are then investigated by a comparative hydrodynamic analysis of the expansion rate up to very high voidage values of such beads in a 1-cm vs. 10-cm internal diameter columns. Terminal velocity conditions are also investigated to compare values with extrapolated expansion properties.

2. Materials and characterization methods

The planned tests required the laboratory preparation of alginate beads, use of appropriate saline solution to store and fluidize the beads and an extensive set of characterization methods, as described below.

2.1. Alginate preparation

The alginate beads are prepared by the process of alginate drops polymerization in calcium chloride, a careful procedure known to produce relatively monodisperse particles. To prepare the alginate solutions, alginate powder (MANUCOL® LKX 50DR, FMC BioPolyme) is dissolved in a sterile saline solution (154 mM NaCl solution buffered with 10 mM Hepes, pH 7.4). The mixture is then filtered using a 0.2 µm membrane. Alginate suspension solution is extruded as droplets through a gauge system. Droplets are size-controlled using co-axial air flow. The alginate droplets are collected in a calcium chloride gelation bath (154 mM NaCl, 10 mM Hepes, 115 mM $CaCl_2$) wherein they are immerged and reticulated for 15 min. Afterwards, the beads are rinsed twice with sterile saline solution. The inert beads are then stored in the Ringer solution before use, whereas the hepatocytes-containing beads are placed in a culture vessel containing William's E medium (PAN Biotech).

2.2. Ringer solution

Alginate beads, even not encapsulating cells, have been shown to exhibit structural instability unless stored in appropriate solutions. Expansion tests have therefore been carried out using the simplest solution known to ensure stability to the material, i.e. the Ringer solution (6.5 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$ and 0.29 g $NaHCO_3$ per litre of water). For the 10-cm diameter set-up, 200 l of solution based on purified water (Milli-Q Merck Millipore) are prepared.

2.3. Characterization techniques: size

The particle size distribution of the alginate beads is measured by laser diffraction using the Malvern Mastersizer 2000, with Ringer solution as dispersion medium. Sauter's average (volume-to-surface, or $d_{3,2}$) diameter is calculated according to the distribution. Distributions are measured for two samples, each one subjected to three evaluations, and the average values are reported.

2.4. Characterization technique: density

As shown below, the hydrodynamic characteristics of the fluidized bed bioreactor is very sensitive to density. Therefore, density measurements are separately carried out for inert beads and beads encapsulating hepatocyte spheroids.

2.4.1. Inert beads

The density of inert alginate beads is calculated by separate measures of the mass and volume of approx. 8 ml (bulk) of beads. The same sample is weighted by laboratory balance; the net bead volume is then precisely evaluated by helium pycnometry (Quantachrome Ultrapycnometer 1000). The volume measurements were repeated five times and the average value and standard deviation calculated.

In addition, four samples of alginate beads are prepared for a simpler evaluation of inert bead density to compare with the beads filled with hepatocyte spheroids. To this purpose, the density is calculated by separate measures of mass and volume using laboratory balance and graded cylinder. In this case a bulk volume of 3 ml of weighed alginate beads in 20 ml of solution (Ringer and William's E medium) is used.

2.4.2. Beads with cellular spheroids

For density measurements of alginate beads with spheroids, a set of alginate beads is synthesized with encapsulated spheroids under sterile conditions. The number of alginates beads is divided into three parts (each containing about 2 million cells) for three different tests at day 0, day 5 and day 7. The density is measured by the same technique as for inert beads.

2.5. Characterization technique: swelling

Transient swelling characterization of inert alginate beads, important for its effect on density and the related hydrodynamic implications, is investigated after storage in Ringer solution and William's E medium. Rather than statistical distributions on many particles, bead swelling requires observation of the same particles over time. Thus, the evolution of the beads size is analyzed under an inverted light microscope equipped with phase contrast (Leica DMI 6000B, Leica, Wetzlar, Germany). For each average datum, the diameter of 20 alginate beads is measured with the help of an image analysis and processing software (LAS AF software). Two batches, one per medium, are prepared. The size of beads in the batch is analyzed under the microscope every 2 h for 6 h and then every 24 h until steady state conditions are achieved.

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