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A method for preparation of hydrogel microcapsules for stem cell bioprocessing and stem cell therapy

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ABSTRACT

A method for the preparation of suspension culture microcapsules used in the bioprocessing of human mesenchymal stem cells (hMSCs) is reported. The microcapsules are prepared from a semi-synthetic hydrogel comprising Pluronic®F127 conjugated to denatured fibrinogen. The Pluronic-fibrinogen adducts display a lower critical solubility temperature (LCST) at ~30 °C, thus enabling mild, cell-compatible physical crosslinking of the microcapsules in a warm gelation bath. Cell-laden microgels were prepared from a solution of Pluronic-fibrinogen hydrogel precursor and hMSCs; these were cultivated for up to 15 days in laboratory-scale suspension bioreactors and harvested by reducing the temperature of the microcapsules to disassemble the physical polymer network. The viability, proliferation and cell recovery yields of the hMSCs were shown to be better than photo-chemically crosslinked microcapsules made from a similar material. The cell culture yields, which exceeded 300% after 15 days in suspension culture, were comparable to other microcarrier systems used for the mass production of hMSCs. The simplicity of this methodology, both in terms of the cell inoculation and mild recovery conditions, represent distinct advantages for stem cell bioprocessing with suspension culture bioreactors.

1. Introduction

An emerging approach in regenerative medicine involves the replacement of damaged cells with healthy ones that have the potential to restore function of damaged tissues [1]. In this context, stem cells are anticipated to become the ideal cell source for this approach [2]. They have shown efficacy in a variety of experimental models of tissue regeneration, including bone, cartilage, fat and muscle [3–9]. Hematopoietic stem and progenitor cells (HSC) are already routinely used in the clinic [10]; and pluripotent human mesenchymal stem cell (hMSCs) therapies are expected to provide far more treatment options [9,11,12]. This potential of cultured stem cells has already begun to materialize into clinical products poised to reach the market in just a few short years [13–16].

Given the high priority for commercialization of cell therapy in general and stem cell therapy in particular, one of the toughest tasks facing this field is how to generate the large numbers of cells required for the eventual treatment of large patient populations. Most research-stage projects working with stem cells can generate only a limited number of cells that support proof-of-concept clinical trials [17]. With technologies based on autologous cells, it is not clear how theses cells can be efficiently extracted, expanded, differentiated in vitro, and then delivered back into the patient. Regardless of the advanced stages of some stem cell technologies, a commercial medical delivery infrastructure for stem cell therapies is still very much in its infancy. The realization of stem cell therapies will therefore require robust, efficient and reproducible bioprocessing methodologies. In this context, suspension culture bioreactors are highly favoured in process scale-up because established culture conditions in lab-scale can often be transferred to much higher volumes with relative ease [18].

A key factor used to control stem cell growth in vitro is the matrix provided for cell attachment. With the exception of HSC, which are generally expanded without a cell attachment matrix, most other stem cell types have been isolated under conditions dependent on surface adherence. Amongst these are hMSCs, which are adherent cells that require culture surface enlargement to ensure efficient and reasonable mass production. Hence, the expansion of anchorage-dependent cells on two-dimensional (2D) substrates is a central challenge in bioreactor design. Moreover, the considerable cost with respect to consumables, labour and time as well as the inherent variability in manual processes of 2D culture not only make this option unattractive, but also render it commercially unviable. In this regard, automation

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and the use of an efficient bioprocessing paradigm are imperative for the creation of successful clinical products.

A variety of three-dimensional (3D) cell carriers for the suspension culture of hMSCs have been developed during the past few years [19,20]. When used for 3D cell cultivation, these platforms can also provide a physical support for cell attachment, proliferation and differentiation [21]. Both synthetic and biological polymers have been studied in order to find the ideal scaffold material, which integrates structural properties of synthetic polymers with bio-functionality of natural extracellular matrix (ECM) constituents [22–26]. For example, ECM proteins or polysaccharides such as alginate, hyaluronic acid, collagen, and fibrin have been used as culture scaffolds of hMSCs [24–27]. Biodegradable protein hydrogels that directly encapsulate cells within a 3D microenvironment (i.e. microcapsules) have many advantages [19,20,28], including easy inoculation, precise control of the cell microenvironment, and straightforward cell recovery. Additionally, microencapsulation of cells in these biomaterials can mimic the body's extracellular environment better, and capture the ability of cells to proliferate in this more natural milieu [29,30]. Moreover, such scaffolds can provide physical protection and better control of biodegradation [29,30], two very important features that are beneficial in the often-harsh hydrodynamic environment of suspension bioreactors.

When culturing MSCs in microcapsules, another factor that should be considered from the scaffold material is the influence of the material modulus on the cultured cells [31,32]. Previous studies demonstrated that MSCs are highly responsive to matrix modulus, and can often show unnatural behavior when they are removed from their tissue microenvironment [33]. Culturing MSCs in 3D embryoid bodies, for example, enhances chondrogenesis [34], suggesting that MSCs may be guided to this and other differentiation pathways during their suspension culture using simple modifications to the microcapsule properties. Finding a way to guide stem cell fate determination using the stiffness of culture substrates is a focus of much research, and will likely become essential aspect of future suspension culture paradigms for stem cell bioprocessing.

Premised on these concepts, our group has recently developed microcapsules made from a bioactive semi-synthetic hydrogel. The biomaterial is made by conjugating the Pluronic®F127 to fibrinogen [35–37]. The Pluronic®F127 is a stimuli-responsive synthetic block copolymer that exhibits lower critical solution temperature (LCST) behavior [38,39]. When Pluronic®F127 is conjugated to fibrinogen, the semi-synthetic precursor retains bioactivity from the fibrinogen and LCST properties from the Pluronic®F127 [35,37]. In the current study, we exploit this material for developing bioprocessing methodologies for hMSCs, through their microencapsulation in hydrogel microcapsules and subsequent cultivation in suspension bioreactors. The fast LCST gelation, combined with photo-initiated chemical crosslinking, and multi-functional protein-like bioactive domains provides the features that help to facilitate the cell inoculation, control the cultivation microenvironment, and expedite the recovery of the cells from the microcarriers at the end of the culture period. This combined approach aims to provide a routine, efficient, and scalable solution for hMSC bioprocessing in suspension cell culture systems.

2. Materials and methods

2.1. Synthesis of Pluronic®F127-DA and Pluronic®F127-Fibrinogen adducts

Temperature-responsive bioactive hydrogels were made from fibrinogen conjugated to Pluronic®F127 (F127), a poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO)-PEO tri-block co-polymer that exhibits lower critical solubility temperature (LCST) properties. The F127 was end-functionalized with acryl groups to form F127-diacrylate (F127-DA), and then reacted with denatured bovine fibrinogen (Bovagen, Melbourne, Australia) via a Michael-type addition reaction to form the Pluronic-fibrinogen biosynthetic copolymer [37]. A 7 mg/ml fibrinogen concentration was used for making Pluronic-fibrinogen hydrogels. Poly (ethylene glycol)-diacrylate (PEG-DA) was prepared from 10 kDa PEG-OH (Merck) as described elsewhere [40].

2.2. Shear modulus measurements

Mechanical properties of the Pluronic-fibrinogen were characterized with a strain-rate controlled shear rheometer (AR-G2, TA Instruments, Delaware, USA) equipped with a Peltier plate temperature controlled base, an overhead UV curing assembly and a transparent geometry. Time-sweep oscillatory tests were performed in 50 mm parallel-plate quartz geometry using 600 μL of Pluronic-fibrinogen hydrogel precursor solution containing 0.1% w/v Irgacure®2959 photoinitiator (Ciba, Basel, Switzerland). In order to find the linear viscoelastic region of the time-sweep tests, oscillatory strain (0.1–10%) and frequency sweeps (0.1–10 Hz) were conducted. Rheology experiments were performed at 2% strain and 1 Hz.

2.3. Cell culture and microencapsulation procedure

Human mesenchymal stem cells (hMSCs, Lonza, Basel, Switzerland) were purchased and expanded in MSC growth medium (MSCGM, Lonza) containing 1% Pen-Strep (Kibbutz Beit Haemek, Israel) for 4 passages (12–14 days of expansion for each passage, corresponding to 4 doublings from P4 to P8). Cells were harvested and removed from 2D Flasks (Nunc, New York, USA) using trypsin EDTA solution B (Biological Industries). Centrifugation of the cells was performed at 1000 RPM for 5 min at room temp to obtain the hMSC pellet for proliferation experiments. In order to prepare the microcapsules, pelleted hMSCs were mixed with Pluronic-fibrinogen hydrogel precursor containing 0.1% Irgacure®2959 photoinitiator (Ciba, Basel, Switzerland). Two types of hydrogel microcapsules were prepared from the cell/polymer solution: a chemically crosslinked version and a physically crosslinked version.

For physically crosslinked microcapsules, 400 μL cell/polymer mixture was dripped through a 30 gauge syringe needle into a warm (37 °C) gelation bath containing continually stirred culture medium (Supplementary video 1), forming beads with a diameter ranging from 500 to 1000 μm. The typical microcapsule volume was 0.25–0.5 μL (the polydispersity of the bead size and volume was not characterized). For the chemically crosslinked microcapsules, the cell/polymer solution was supplemented with PEG-DA (at a concentration of 0.2–0.5% w/v) and similarly dripped onto a super-hydrophobic surface at RT, followed by UV-light-activated photopolymerization (365 nm, 4–5 mW/cm^2) for 1.5 min. The procedure created droplets of 0.25–0.5 μL and diameter of 500–1000 μm. The hMSC microcapsules (physically or chemically crosslinked) were cultured in laboratory-scale stirred-flask bioreactors (500 ml) for up to 15 days using expansion MSC growth medium (Lonza). The microcapsules were stirred at 1 RPM to ensure proper transport in the reactor volume. The cells were harvested from the hydrogel microcapsules on days 1, 3, 7 and 15, either by cooling down to 4 °C for physically crosslinked microcapsules (Supplementary video 2) or by using collagenase incubation for the chemically crosslinked microcapsules, as detailed below.