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Therapeutic bioactive microcarriers: Co-delivery of growth factors and stem cells for bone tissue engineering

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ABSTRACT

Novel microcarriers made of sol–gel-derived bioactive glasses were developed for delivering therapeutic molecules effectively while cultivating stem cells for bone tissue engineering. Silica sols with varying concentration of Ca (0–30 mol.%) were formulated into microspheres ranging from 200 to 300 μm under optimized conditions. A highly mesoporous structure was created, with mesopore sizes of 2.5–6.3 nm and specific surface areas of 420–710 $m^2 g^{-1}$, which was highly dependent on the Ca concentration. Therapeutic molecules could be effectively loaded within the mesoporous microcarriers during microsphere formulation. Cytochrome C (cyt C), used as a model protein for the release study, was released in a highly sustainable manner, with an almost zero-order kinetics over a period of months; the amount released was ~2% at 9 days, and 15% at 40 days. A slight increase in the release rate was observed in the microcarrier containing Ca, which was related to the dissolution rate and pore size. The presence of Ca accelerated the formation of hydroxyapatite on the surface of the microcarriers. Cells cultured on the bioactive microcarriers were well adhered and distributed, and proliferated actively, confirming the three-dimensional substrate role of the microcarriers. An in vivo study performed in a rat subcutaneous model demonstrated the satisfactory biocompatibility of the prepared microspheres. As a therapeutic target molecule, basic fibroblast growth factor (bFGF) was incorporated into the microcarriers. A slow release pattern similar to that of cyt C was observed for bFGF. Cells adhered and proliferated to significantly higher levels on the bFGF-loaded microcarriers, demonstrating the effective role of bFGF in cell proliferative potential. It is believed that the developed mesoporous bioactive glass microspheres represent a new class of therapeutic cell delivery carrier, potentially useful in the sustainable delivery of therapeutic molecules such as growth factors, as well as in the support of stem cell proliferation and osteogenesis for bone tissue engineering.

1. Introduction

The release of specific signaling molecules from scaffolding materials to elicit desired cellular reactions is one of the key strategies to significantly enhance the regenerative capacity of synthetic biomaterials for use in bone tissue engineering. These signaling molecules can either be eluted to the cells that are supported upon the scaffolds for ex vivo cultivation appropriate for bone engineering, or can be supplied to the surrounding bone defects when directly implanted in vivo [1]. For either case, the signaling molecules should ideally be released in a controlled manner, and for certain durations, in order to optimize cellular propagation and/or osteogenic differentiation, as well as to aid in vivo vascularization and bone formation. Hence, one of the primary requirements of scaffolds is the sustained and controllable release of therapeutic molecules, while enabling their effective and safe incorporation into the scaffold structure.

Microspherical scaffolds, namely, microcarriers, have gained great interest as three-dimensional (3-D) substrates for the 3-D cultivation and expansion of tissue cells. Frequently allowed to rotate in cell suspensions in vitro, so as to aid cellular anchorage, the isotropic 3-D spherical substrates provide homogeneous sites for cellular recognition, distribution and multiplication [2]. Subsequently, cell-loaded microcarriers can be delivered to the defects of concern, with a delivery capacity that is controllable based on the size of the microcarriers. Furthermore, the cell–carrier constructs are possible as injectable tissue engineering devices,

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effectively filling defects [3]. In fact, the micro-spherical particles, which may be a few to hundreds of micrometers in diameter, have been extensively researched for the delivery of therapeutic molecules, including drugs, hormones and growth factors [4,5]. Along with this biomolecular delivery use, the cellular delivery potential has thus been of great interest for the ex vivo culture of stem cells and engineering of tissues, including bone.

Among other things, providing therapeutic roles to the microcarriers is of great merit in regulating the behavior of stem/progenitor cells to be supported, ultimately for bone tissue engineering, such as rapid cellular engulfment, increased cell population, stimulation to osteogenic lineage specification and/or achieving highly vasculature tissues [2]. When microcarriers are used for the delivery of therapeutic molecules, cells can be made to undergo osteogenesis during ex vivo cultivation prior to implantation and, further, to play beneficial roles in the regeneration process of bone tissue in vivo, after implantation.

Therefore, the current aim is to develop therapeutic microcarriers effective for bone tissue engineering that allow 3-D cultivation of stem cells in vitro, while incorporating and releasing therapeutic molecules ultimately to aid their osteogenesis and in vivo bone formation. Here, the present authors propose a "bone-bioactive" inorganic composition composed of silica-based bioactive glass (SBG). The SBG is made through a sol–gel process under room temperature and aqueous conditions [8–12]. Different ions could be easily incorporated into the silica sol–gel glass network, and the addition of calcium greatly improved the bioactivity and hydrolytic degradation. Natural polymers such as gelatin and collagen have also been added, to improve the mechanical properties and cellular responses [13–15]. Importantly, the sol–gel process enables the introduction of therapeutic molecules because of the mild processing conditions. Several studies have demonstrated the effectiveness of the sol–gel glass network in capturing drugs and proteins and their release for long periods [16–20].

Here, the SBG composition is used in the preparation of therapeutic microcarriers, which are effective for bone tissue engineering. The SBG composition has merits over polymer-based compositions, particularly for therapeutic purposes, which can limit the incorporation of biomolecules. Synthetic polymers are generally produced in organic solvents or surfactant-mediated conditions, requiring vigorous washing steps, while natural polymers require crosslink steps to stabilize the structure, although they may be processed in aqueous solutions [2]. Moreover, the SBG composition based on a sol–gel process has a number of merits compared with conventional bioceramics, such as calcium phosphates and melt-derived bioglasses, that are generally prepared at high temperatures [6,7]. Owing to the nature of the sol–gel reaction, i.e., hydrolysis and polycondensation, sol–gel processed BG spherical particles self-harden in a structurally and chemically stable manner, eliminating further crosslinking steps.

Another intriguing and beneficial point of SBG is the sol–gel-derived mesoporous structure, where the mesoporosity and mesopore geometry are tuned to take up a large quantity of and selective therapeutic molecules and, subsequently, to control their release behavior [16–18]. Furthermore, the silanol groups present on the surface are hydrophilic and easily inducible for calcium and phosphate ions to produce calcium phosphate crystals, which are recognized as "bone-bioactive" materials [21], and therefore SBG is considered to be a proper reservoir for therapeutic molecules and 3-D substratum for cellular reactions, particularly those required for bone regeneration.

In the present study, SBG microcarriers with bone-bioactive and self-hardening properties are prepared. The ability to populate stem cells in vitro, as well as to allow favorable reactions in vivo, is briefly assessed. Model experiments on incorporating therapeutic protein molecules within the structure and releasing them sustainably, as well as the accompanying biological effects, are also described. These studies support the further use of novel therapeutic microcarriers in bone tissue engineering.

2. Materials and methods

2.1. Preparation of SBF microcarriers

Tetraethyl orthosilicate (TEOS, $C_8H_{20}O_4Si$, 98%, Sigma–Aldrich) (10 ml) was mixed with 0.1 M HCl (2.4 ml), with the addition of deionized water to form an acid catalyzed sol. The molar ratio of total water (including the $CaCl_2$ and the HCl water) to TEOS was 8. The microspheres were doped with calcium ions by incorporating specific amounts of calcium chloride ($CaCl_2$, Sigma–Aldrich) into the solution. The molar percentage of doped calcium ranged from 0 to 30. Once the sol was obtained, it was cooled down in a water bath at 4 °C. Afterwards, 0.08 M ammonium hydroxide (NH_4OH, 28.0% NH_3 in water, $\geqslant$99.99% metal basis, Sigma–Aldrich) was added dropwise to the sol with agitation. The pH was adjusted to 5–5.5, and 5 ml of the sol was then added dropwise to 100 ml of olive oil, and was stirred at 95 rpm to allow gelation. Gelled microspheres were gathered after precipitation at the bottom of the flask, vacuum filtered, rinsed with water and ethanol, and left overnight to dry. Microcarriers with different Ca concentrations were designated as "0Ca", "10Ca", "20Ca" and "30Ca" for 0%Ca–100%Si, 10%Ca–90%Si, 20%Ca–80%Si and 30%Ca–70%Si, respectively.

2.2. Characterizations of microcarriers

The size distribution of the microspheres was characterized by means of laser diffraction granulometry (Malvern, APA5001SR). Specific surface area as well as the pore size distribution was quantified using a Quadrasorb SI automated surface area and pore size analyzer (Quantachrom Instruments). Samples were degassed under vacuum at 300 °C for 12 h prior to analysis. The specific surface area was determined according to the Brunauer–Emmett–Teller (BET) method. The pore size distribution was determined from the N_2 desorption branch of the N_2 adsorption–desorption isotherms obtained on the basis of the density functional theory method. Total pore volume was calculated from the amount adsorbed at a maximum relative pressure (P/P_0). Scanning electron microscopy (SEM) images were taken to observe the morphology and particle size (JEOL JSM-6510). X-ray diffraction (XRD; Philips MRD) was conducted to analyze the phases. X-rays were generated at 40 mA and 40 kV, and data were obtained at diffraction angles (2θ) from 4 to 45°, with a step size of 0.02° and a scanning speed of 5° min^{-1}. The chemical bond status in the microspheres was analyzed by Fourier transform infrared spectrometry (FTIR; Varian 640-IR). The ζ potential of the microspheres was measured using a Zetasizer Nano ZS laser Doppler electrophoresis instrument (Malvern Instruments). The ζ potential was measured five times at 25 °C with an applied field strength of 20 V cm^{-1} (where each data point is the average of 40 runs), and the mean ± standard deviation ($n = 5$) were calculated. The instrument automatically calculated electrophoretic mobility (U), and ζ potential according to the Helmholtz–Smoluchowski equation: $\zeta = U\eta/\varepsilon$, where ζ is the zeta potential, U is the electrophoretic mobility, η is the dispersing medium viscosity, and ε is the dielectric constant.

2.3. In vitro apatite forming ability

The in vitro apatite forming ability of the microcarriers was evaluated through the formation of apatite on the surface of the microspheres when immersed in a simulated body fluid (SBF) solution. One hundred milligrams of microcarriers with diameters