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Polycaprolactone porous template facilitates modulated release of molecules from alginate hydrogels

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ABSTRACT

The development and study of advanced methods to encapsulate and provide a controlled release of drugs and bioactive agents provide the opportunity to deliver active factors in situ. This acute delivery requires systems to be able to precisely control the release rate, however this has been challenging to achieve using a single component system. We developed novel Polycaprolactone (PCL) porous templates to control the release of encapsulated alginate hydrogel containing water-soluble molecules of different molecular weights. The presence of molecule-loaded alginate within the PCL porous templates was confirmed by infrared spectroscopy, fluorescence imaging and scanning electron microscopy. The influence of the molecular weight of the encapsulated molecule was investigated. The release profile of PCL-alginate displayed significant improvement on slowing the release of molecules of different molecular weight when compared with alginate alone. Based on the results, we can suggest that PCL porous templates can be a promising candidate to form composite delivery systems carrying high molecular weight hydrophilic agents for tissue engineering and drug delivery applications.

1. Introduction

Tissue engineering aims to provide alternatives to restore functionality of body parts and effective ways to treat diseases [1]. In order to develop such platforms it is crucial to have molecules that can improve, drive or promote specific environments [2]. It has been shown that the formation and regeneration of tissues can be enhanced by the localised delivery of bioactive agents (e.g. growth factors). Through encapsulation methods, the structure and biological activity of such bioactive molecules can be preserved to be released at the target site [3]. The formation of suitable carriers depends largely on the characteristics of the cargo molecule such as the water affinity and the molecular weight. Ideally the carrier should also be biocompatible and biodegradable.

Hydrogels have been largely studied to encapsulate compounds [4–6]. A common example of these materials is alganic acid or alginate, which is derived from brown seaweed and composed of 1–4 linked α-L-guluronic (G) and β-D-mannuronic (M) acid. Alginate can form gels by cross-linking a pair of G-blocks in the polymer chain using divalent cations (e.g. Ca^{+2}, Ba^{+2}). The formed alginate hydrogels can contain up to 90% water resulting in highly biocompatible networks, which has led to their use as tissue engineering scaffolds [7–9]. The dispersal of aqueous soluble agents in the alginate solution enables gels to be formed encapsulating drugs and bioacitve species such as growth factors, proteins and living cells [10–13]. The major drawback of alginate hydrogels in delivering biologicals, is that alginate hydrogels degrade rapidly and present an initial high burst release of the cargo. This characteristic makes unsuitable for long-term delivery.

In contrast, a synthetic polymer such as polycaprolactone (PCL), has a slow hydrolysis degradation profile (months to years) making it more suitable for longer-term release applications. Critically, the degradation products are cleared by the natural physiological pathway [14]. Due to its high biocompatibility, PCL is currently used for medical applications such as sutures and adhesion barriers for surgery [15]. In tissue engineering PCL has been 3D printed and electrospun to form scaffolds able to host different cell lines during in vivo and in vitro experiments [16,17]. In the field of drug delivery, PCL has been employed to encapsulate drugs with poor aqueous solubility such as Sagopilone or Paclitaxel [18,19]. The degree of encapsulation and the mechanism of bioactive molecules and drugs entrapment are dependent on hydrophobic-hydrophobic or hydrophilic-hydrophilic interactions among the molecules and the carrier [20]. Due to the hydrophobic nature of PCL,

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its use has been limited to non-water soluble drugs.

The carrier needs to be tailored towards the specific delivered substance. The same carrier can show a different release profile depending on the encapsulated substance according to the cargo's physical and chemical characteristics [21,22]. For instance, the molecular weight of the encapsulated molecule can play an important role in the release profile. [23,24]. Previous studies have investigated strategies to control the release from hydrogels that encapsulate bioactive agents of specific molecular weights through the use of composites. A common approach is to create a blend between two or more hydrogels (e.g. alginate and chitosan) which aims to slow the release by forming high crosslink density hydrogels [25]. Modification of the carrier hydrogel has also been investigated by attaching a polypeptide, such as RGD, that can potentially bind to the encapsulated molecule to limit passive diffusion [26]. Another strategy is to create a system using a hydrogel coated by a synthetic polymer creating a layered structure in which the release is dependent on the bulk degradation of the coat polymer [27].

In this work, we propose a PCL porous template to preserve molecules encapsulated. The porous carrier allows the integration of an alginate solution loaded with molecules which can be crosslinked to form a hydrogel integrated within the template. The PCL porous template aims to reduce the burst and to prolong the release without compromising the sustained release. To test this hypothesis, we developed a strategy to encapsulate water-soluble molecules using a simple method. The encapsulation and release of fluorescein isothiocyanate (FITC)-dextran sulphate from PCL-alginate was then compared to alginate hydrogels for a range of molecular weights testing the flexibility of the system.

2. Materials and methods

2.1. Materials

All materials were analytic grade and purchased from Sigma-Aldrich unless otherwise specified. Alginic acid sodium salt (A2033), calcium chloride (FW: 147, C-5080), 2-Propanol (33539), polycaprolactone (Mnca. 80,000 GPC. 44,074-A), chloroform (C2432), sodium hydroxide (221465), phosphate buffered saline (P4417), sodium citrate (C1909). FITC-Dextran sulphate (MW: 4000, 40,000 and 500,000 g/mol. BCBS033, 51,923 and 78,331).

2.2. Microspheres fabrication

2.2.1. PCL-alginate microspheres

PCL porous templates were prepared via oil-in-water emulsion and solvent evaporation method using a previously reported protocol slightly modified [28]: 3 g of PCL (Mn: 80,000) was dissolved in 12 ml of chloroform (oil phase). The oil phase was emulsified (homogenizer, Ika Ultra-Turrax 25) at 16000 rpm with a water phase (formed by 50 ml of sodium hydroxide solution (0.5 M)). The solution was kept at 37°C for 24 h in a water bath. Evaporation of the organic solvent was achieved using magnetic stirring (600 rpm) at 60°C (Fig. 1A). Next, the solution was stored overnight at 4°C, then at −80°C for 5 h before being freeze dried to obtain the PCL porous templates.

After obtaining the PCL porous templates, an additional water-in-oil emulsification process was used to integrate the alginate (Fig. 1B). Five millilitres of alginate solution 2% (*w/v*) containing 0.01 g of FITC-dextran (MW: 4 kDa, 40 kDa or 500 kDa) was added to the PCL templates and stirred overnight (1500 rpm) next, 50 ml of paraffin oil was added and mixed using a homogenizer (model: Ika Ultra-Turrax 25) at 16000 rpm for 1 min. Then, 3 ml of $CaCl_2$ (0.5 M) was drop-wise added to the emulsion under magnetic stirring (1500 rpm) for 2 h. Isopropanol (58 ml) was added to harden the spheres. The formed PCL-alginate microspheres were collected by vacuum filtration and washed with isopropanol 3 times.

2.2.2. Alginate microspheres

Microspheres were prepared by an water-in-oil emulsion method with a post $CaCl_2$ crosslinking process. Briefly: 5 ml of alginate 2% (*w/v*) solution containing 0.01 g of FITC-dextran sulphate (mw: 4 kDa, 40 kDa or 500 kDa) was mixed with 50 ml of paraffin oil using a homogenizer (model: Ika Ultra-Turrax 25) at 16000 rpm for 1 min. Then, 3 ml of $CaCl_2$ (0.5 M) was drop-wise added to the emulsion under magnetic stirring (1500 rpm) for 2 h. Isopropanol (58 ml) was added and maintained under magnetic stirring for 20 min to harden the spheres. The formed microspheres were collected by vacuum filtration and washed at least 3 times with isopropanol to remove the oil phase.

2.3. Characterisation methods

2.3.1. Morphology

The surface of the structures was observed and imaged by scanning electron microscope (SEM, SUPRA TM-40VP- GEMINI, ZEISS). Freeze-dried samples were sputter-coated with gold for 45 s (K975X, EMITECH) at an accelerating voltage of 3 kV.

2.3.2. Particle size

The particle size and size distribution was determined by light scattering (Partica Laser Scattering LA-950 V2). The spheres were dispersed in 1 ml of deionized water and 150 μl of the particle suspension was used to measure the particle size. The refractive index of the dispersing medium was calculated based on the observations from an Abbe refractometer at room temperature (25°C).

2.3.3. Chemical composition

The chemical structure of freeze-dried samples was analised by Fourier Transform infra red (FTIR) spectrometer (Nicolet i55, iD5 ATR, Thermo Scientific). The IR spectra represent the mean of 50 scans at wavelength range of $500 - 4000\ cm^{-1}$.

2.3.4. Mass loss

Samples ($n = 3$) of PCL porous templates, PCL-alginate and alginate microspheres were incubated at 37°C in PBS. The samples were collected from the PBS at given times, blotted against filter paper to absorb PBS excess and stored at −80°C. After the final collection, the samples were weighted. Mass loss calculations were obtained using Eq. (1) where *ML* is the mass loss percentage, *Wi* is the initial weight of the spheres and *Ws* the weight after incubation. The mass loss was reported as the average obtained for the three samples.

$$ML(\%) = ((Wi - Ws)/Wi) * 100 \quad (1)$$

2.3.5. Encapsulation efficiency

The encapsulation efficiency (EE%) of spheres was performed by dissolving microspheres prepared with a known amount of FITC-dextran sulphate. Sodium citrate was used to dissolve the spheres. The solutions ($n = 3$) were measured using a fluorescent plate-reader (excitation: $\lambda max = 495$ nm, emission: $\lambda max = 525$ nm). The results were calculated using the calibration curves previously prepared using known concentrations of FITC-dextran sulphate and fluorescent units (correlation factor = 0.99). The encapsulation efficiency was calculated using Eq. (2), where *EE* is the encapsulation efficiency percentage, *M* is the amount of FITC-dextran sulphate obtained from the microspheres and *Mo* is the amount initially added during fabrication.

$$EE(\%) = (M/Mo) * 100 \quad (2)$$

2.3.6. Swelling ratio

The swelling capacity of dried spheres was measured using a gravimetric method. Freshly prepared microspheres were freeze-dried and weighted. The dried microspheres were immersed in PBS (pH 7.4, 37°C). Samples ($n = 3$) were retrieved at determined time points,