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Alginate-based hydrogels with improved adhesive properties for cell encapsulation

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ABSTRACT

Hydrogel-based biomaterials are ideal scaffolding matrices for microencapsulation, but they need to be modified to resemble the mechanical, structural and chemical properties of the native extracellular matrix. Here, we compare the mechanical properties and the degradation behavior of unmodified and modified alginate hydrogels in which cell adhesive functionality is conferred either by blending or covalently cross-linking with gelatin. Furthermore, we measure the spreading and proliferation of encapsulated osteoblast-like MG-63 cells. Alginate hydrogels covalently crosslinked with gelatin show the highest degree of cell adhesion, spreading, migration, and proliferation, as well as a faster degradation rate, and are therefore a particularly suitable material for microencapsulation.

1. Introduction

Cell encapsulation – the immobilization of cells in polymeric hydrogels – is a promising technique in tissue engineering [1]. Hydrogels can act as a semipermeable membrane, which protect the encapsulated cells from the host's immune system while allowing for the bidirectional diffusion of oxygen, nutrients and waste. Moreover, hydrogels attenuate the mechanical stress and friction not only on encapsulated cells but also on adjacent tissue upon transplantation [1–4]. The material of choice for many cell encapsulation applications is alginate because of its biocompatibility and rapid ionic gelation property with divalent cation [5,6]. Alginate represents a family of anionic polysaccharides extracted from brown algae or bacteria. They are linear unbranched copolymers composed of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers [7–9]. The fraction and sequence of M- and G-monomers vary with the origin of alginate and contribute to different chemical and physical properties [10]. When the G-blocks of neighboring polymer chains are linked with each other through divalent cation bridges, for example Ca^{2+}, alginate forms a hydrogel with viscoelastic properties [11]. However, alginate does not promote cell adhesion and proliferation due to the absence of cell adhesion motifs. It has been shown in previous studies that cell adhesion to alginate hydrogels can be achieved by modification of alginate through functionalization with gelatin [12,13]. Gelatin is a biodegradable protein, produced by denaturation of collagen, which transforms the triple helix structure of collagen into a random coil structure [14] and thereby exposes the cell adhesion motif RGD (Arg-Gly-Asp) of collagen [15,16].

In a previous study, we established that alginate hydrogels that were functionalized by crosslinking with gelatin promoted better adhesion, proliferation, and migration of encapsulated osteoblast-like MG-63 cells compared to pure alginate hydrogels or to hydrogels functionalized with the specific integrin binding sequence RGD [12]. However, it has remained an open question whether the presence of gelatin on its own, e.g. by blending with alginate, would be sufficient for cell adhesion, migration, and proliferation, or whether gelatin must be crosslinked with alginate. Furthermore, it is unknown if the presence of gelatin speeds up the degradation behavior of the hydrogel over time and thereby promotes cell migration and proliferation. Finally, it is unknown how stably the gelatin is bound or contained in the hydrogel, or how quickly it is released over time into the surrounding medium.

In this study we compared the behavior of osteoblast-like MG-63 cells in two alginate hydrogel systems that were functionalized with gelatin either by covalent crosslinking of alginate di-aldehyde and gelatin (ADA-GEL-x), or by simple blending of alginate and gelatin prior to polymerization (ALG-GEL-b). We embedded the

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cells in hydrogel capsules of 800 μm diameter, and characterized cell viability, mitochondrial activity, spreading morphology and hydrogel degradation over a time course of up to 28 days. ADA-GEL-x hydrogels facilitated superior adhesion, proliferation, migration, and morphology of encapsulated cells compared to ALG-GEL-b. Moreover, the gelatin release kinetics was slower in ADA-GEL-x compared to ALG-GEL-b, yet the overall hydrogel degradation rate as evaluated from the decrease in stiffness of microcapsules over time was higher. Thus, we conclude that the better adhesion, proliferation, and migration of encapsulated osteoblast-like MG-63 cells in gelatin-crosslinked alginate hydrogel microcapsules arises as a combination of a stronger binding of gelatin to the hydrogel matrix, and a faster mechanical degradation behavior that better accommodates the proliferation and migration of embedded cells.

2. Materials and methods

2.1. Preparation of functionalized alginate gels

Sodium alginate (sodium salt of alginic acid from brown algae, suitable for immobilization of micro-organisms, guluronic acid content 65–70%) and gelatin (from porcine skin, suitable for cell culture, Type A, Bloom 300) were obtained from Sigma–Aldrich, Germany. Ethanol, ethylene glycol, sodium metaperiodate and calcium chloride dehydrate ($CaCl_2{\cdot}2H_2O$) were purchased from VWR International, Germany. Silver nitrate was obtained from Alfa Aesar, USA.

Sodium alginate was dissolved in PBS, and gelatin was dissolved in ultrapure water at 37 °C. Alginate–gelatin blend material (ALG-GEL-b) was prepared by mixing aqueous solution of gelatin with alginate solution with a volume ratio of 80:20, resulting in a final concentration of alginate and gelatin of 2% (w/v) and 0.5% (w/v), respectively.

Alginate–gelatin crosslinked hydrogel (ADA-GEL-x) was synthesized by covalent crosslinking of alginate di-aldehyde (ADA) and gelatin as described in [2]. Briefly, ADA was synthesized through controlled oxidation of sodium alginate in equal volume of an ethanol–water mixture. Sodium alginate was dispersed in ethanol and mixed with an aqueous solution of sodium metaperiodate (oxidizing agent). The suspension was stirred in dark conditions at room temperature for 6 h. The oxidation reaction was stopped by adding ethylene glycol, and the dispersion was dialyzed for 7 days against ultrapure water (Direct-Q, Merck Millipore, Germany) using a dialysis membrane (molecular weight cutoff of 6–8 kDa; Spectrum Lab, USA) to ensure complete removal of sodium metaperiodate. The absence of sodium metaperiodate in the dialysate was checked by adding 1% (w/v) solution of silver nitrate and was confirmed when no precipitation occurred. Afterwards, the ADA solution was lyophilized to obtain dry ADA, which was then dissolved in PBS to get 5% (w/v) solution. To synthesize ADA-GEL-x, 5% (w/v) aqueous solution of gelatin was mixed with 5% (w/v) solution of ADA. The final concentrations of ADA and gelatin were 2.5% (w/v). 2% (w/v) pure alginate (ALG) solution (PBS as solvent) was used as control.

2.2. Cell culture

Osteoblast-like MG-63 cells (LGC, ATCC, Germany) were used for encapsulation. DMEM culture medium (Dulbecco's modified Eagle's medium, Gibco, Germany) was supplemented with 10 vol.% fetal bovine serum (FBS, Sigma–Aldrich, Germany) and 1 vol.% penicillin–streptomycin (PS, Sigma–Aldrich, Germany). Cells were cultured at 37 °C in 95% relative humidity and 5% CO_2, and passaged using standard protocols.

2.3. Preparation of microcapsules and cell encapsulation

Microcapsules from the three different materials were produced by a pneumatic extrusion technique as described elsewhere [2]. Briefly, the solutions of ALG, ALG-GEL-b and ADA-GEL-x were filled into a cartridge (Nordson EFD, USA) and connected to a high precision fluid dispenser (UltimusTMV, Nordson EFD, USA). Solutions were extruded by applying a controlled pressure, collected in a 0.1 M $CaCl_2$ solution, and kept for 10 min to facilitate ionic gelation. The prepared microcapsules were sieved and washed with ultrapure water. For cell encapsulation, the alginate and ADA solutions were sterilized by filtration through a 0.45 μm filter (Carl Roth GmbH + Co. KG, Germany), and the gelatin solution was sterilized by filtration through a 0.22 μm filter. After that, hydrogels were prepared in sterile condition. Osteoblast-like MG-63 cells were mixed with ALG, ALG-GEL-b or ADA-GEL-x at a concentration of 1×10^6 cells per 1 ml of hydrogel solution, and then extruded into microcapsules as described above. The microcapsules with embedded cells were washed with DMEM and incubated in 95% relative humidity and 5% CO_2 at 37 °C. The cell culture medium was replaced every second day.

2.4. Characterization of hydrogel properties

2.4.1. Mechanical properties

Mechanical properties of hydrogel films were measured with a dynamic-mechanical analyzer (DMTA IV, Rheometric Scientific). Measurements were performed at 25 °C in a dynamic frequency sweep (0.1–20 Hz), at which a sinusoidal deformation of constant amplitude was applied to a hydrogel film of a cylindrical shape with a diameter of 16 mm and a thickness of 1 mm. To measure the degradation kinetics of the hydrogels, the fabricated films from the three different hydrogels were incubated in DMEM at 37 °C in 95% relative humidity and 5% CO_2, and repeatedly measured with DMTA over a time course of 28 days. All measurements were carried out in the linear viscoelastic regime at a strain of $\varepsilon = 0.1\%$. Measurements were recorded in triplicate, and the results are expressed as mean ± one standard deviation.

2.4.2. Protein release

Weighted amounts (50 mg) of hydrogel microcapsules were immersed in 2 ml of Hank's balanced salt solution (HBSS) at 37 °C. At selected times, the supernatant was removed, collected for gelatin release analysis, and 2 ml of fresh HBSS solution was added to the samples. Protein concentration in the supernatant was determined using the Lowry method [17,18], with bovine serum albumin (BSA) as a standard [19]. The absorbance at 750 nm was measured using a UV–Vis spectrophotometer (Specord 40, Analytik Jena, Germany) [13]. The release of gelatin from the samples was calculated as follows:

$$Gelatin\ \ release\,(\%) = \frac{[Gelatin]_{supernatant}}{[Gelatin]_{total}} \times 100$$

where $[Gelatin]_{total}$ is the initial amount of gelatin, and $[Gelatin]_{supernatant}$ is the cumulative amount of gelatin in the supernatant at different incubation time points.

The molecular mass distribution of released protein fragments was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Mini-PROTEAN 3 system, Bio-Rad). Polyacrylamide gels, with a concentration of 10% acrylamide and a thickness of 1 mm, were run at a constant voltage of 120 V. The Prestained Page Ruler marker (Thermo Scientific) was used for calibration. The visualization of the protein bands was performed using silver staining (Pierce Color Silver Stain Kit, Thermo Scientific).