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Mussel-inspired alginate gel promoting the osteogenic differentiation of mesenchymal stem cells and anti-infection

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ABSTRACT

Alginate hydrogels have been used in cell encapsulation for many years but a prevalent issue with pure alginates is that they are unable to provide enough bioactive properties to interact with mammalian cells. This paper discusses the modification of alginate with mussel-inspired dopamine for cell loading and anti-infection. Mouse bone marrow stem cells were immobilized into alginate and alginate-dopamine beads and fibers. Through live-dead and MTT assay, alginates modified by dopamine promoted cell viability and proliferation. *In vitro* cell differentiation results showed that such an alginate-dopamine gel can promote the osteogenic differentiation of mesenchymal stem cell after PCR and ALP assays. In addition to that, the adhesive prosperities of dopamine allowed for coating the surface of alginate-dopamine gel with silver nanoparticles, which provided the gel with significant antibacterial characteristics. Overall, these results demonstrate that a dopamine-modified alginate gel can be a great tool for cell encapsulation to promote cell proliferation and can be applied to bone regeneration, especially in contaminated bone defects.

1. Introduction

Over the past fifty years, research has shown a very wide range of applications of cell encapsulation for a large number of health issues [1–3]. Cell encapsulation demonstrates a very high potential for future tissue engineering applications, since Chang proposed the concept of artificial microencapsulation in 1964 [4]. Immobilization of cells within microspheres or fibers not only provides the cells with immunoprotection, but also acts an ideal way of delivering living cells and a source of cell products such as growth factors, cytokines, and other extracellular matrix molecules to the injured site. This ability of cellular transport provides it with a high potential in treatment of various types of diseases [5]. So far, there have been numerous promising applications of cell encapsulations as a tool for tissue engineering, regenerative medicine and therapy for a large range of diseases, such as cancers [6,7], metabolic or neurological disorders [8,9], heart diseases [10,11], bone defects [12,13], and so on.

Many kinds of materials such as alginate, cellulose sulphate, collagen, chitosan, gelatin and agarose have been investigated for cell encapsulation [14–16]. Among them, alginate has been extensively studied and the most widely used due to its high biocompatibility and ability to form a gel easily and rapidly under very mild conditions [17–19]. Alginates cross-linked with divalent cations, like Ca^{2+} or Ba^{2+} ions, have been used successfully to encapsulate cells and have shown good cell viability results, even after a long-term culture [20,21]. However, alginate has low cell adhesiveness due to its poor protein adsorption for the hydrophilic nature [22]. Therefore, alginate has been blended with chitosan, collagen or gelatin to enhance cell interaction and has showed better properties for cell adhesion and proliferation [23–26]. Other strategies have also been carried out to enhance cell anchorage and interaction within alginate gel. For example, alginate was modified with growth factors or peptides containing a RGD sequence to promote cell viability (*e.g.* cell attachment, migration and differentiation) [27–29].

Recently, inspired by marine mussels' adhesive mechanism, researchers have exploited 3,4-dihydroxyphenylalanine (DOPA), which is believed to contribute to mussels' adhesive property, to enhance the adhesive ability of solid substrates [30,31]. Further studies have used dopamine (the derivative of DOPA) coating on many kinds of substrates, organic or inorganic, to promote cell adhesion [32–38]. Other researchers have also investigated catechol-containing materials as mimics of mussel adhesives to enhance cell viability [39–41]. In addition, the metal-binding ability of catechol has suggested that it can effectively function as an active reducing agent for generating silver nanoparticles. Increasing efforts have been made to use dopamine to reduce nano-silver for antibacterial action [42,43]. Nano-silver has a broad spectrum of antibacterial activity and low toxicity in the body [44], and in addition to that, the procedure to deposit silver nanoparticles on the gel surface is versatile and very facile.

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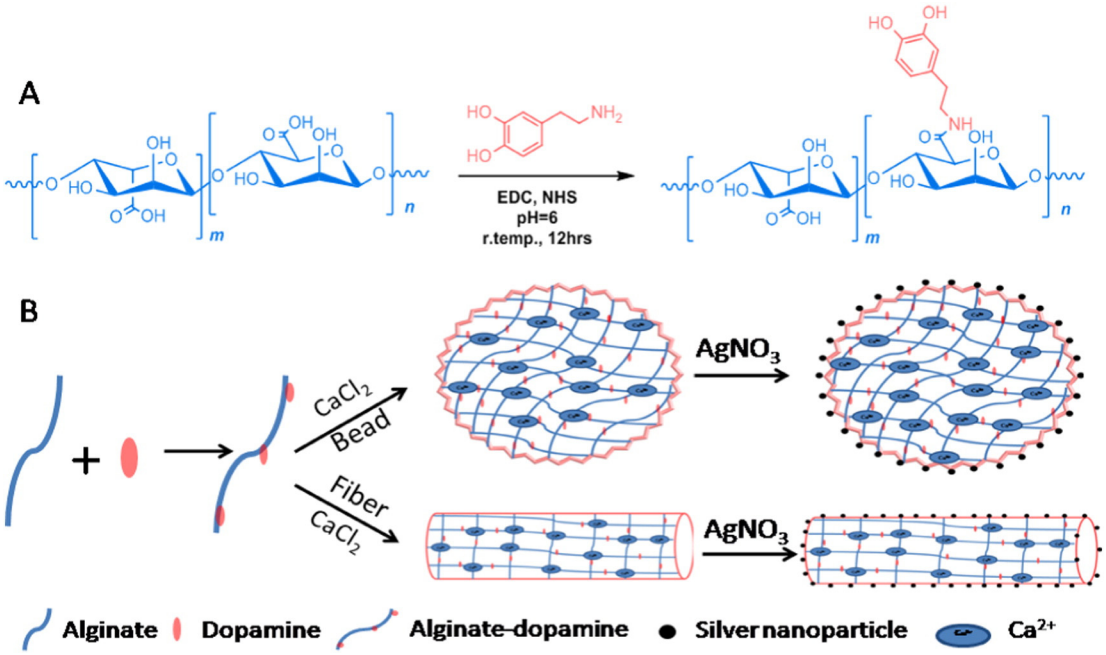

Fig. 1. A: Schematic illustration of the synthesis process of dopamine modified alginate B: Schematic demonstration of the process of dopamine conjugating to alginate, alginate-dopamine bead and fiber formation and deposition of silver nanoparticles on the surface of alginate-dopamine bead and fiber.

In this study, a new strategy for cell encapsulation using alginate is reported which employs modification with dopamine in order to enhance cell viability. At the same time, silver nanoparticles are immobilized on the alginate-dopamine gel surface, utilizing the metal-binding ability of catechol, to achieve an antibacterial effect. The overall goal of this project is to apply nano-silver coated alginate-dopamine encapsulations of bone marrow stem cells for bone regeneration, especially in contaminated sites.

2. Materials and methods

2.1. Materials

1-(3-Dimethylaminopropyl)-3-EthylcarbodiimideHydrochloride (EDC), Alginic Acid Sodium Salt (low viscosity), Dopamine Hydrochloride were all purchased from Alfa Aesar Co. (Ward Hill, MA, USA). *N*-Hydroxy-Succinimide (NHS), Silver Nitrate ($AgNO_3$), Sodium Borohydride ($NaBH_4$), ALP Staining kit were all purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Calcium Chloride Dihydrate ($CaCl_2 \cdot 2H_2O$) and TRIS were obtained from EMD Millipore (Bedford, MA, USA). BODIPY® FL Phallacidin, TO-PRO_3 iodide and Live-Cell staining kit were all purchased from Invitrogen (Carlsbad, CA, USA). The3-(4, 5-Dimethylthiazolyl-2)-2,5-Diphenyltetrazoliumbromide (MTT) cell viability assay kits were from Biotium Inc. (Hayward, CA, USA). TRIzol RNA extract kit was from Ambion (Austin, TX, USA). First Strand cDNA Synthesis kit and SYBR Green qPCR Kit were from Thermo Scientific (Hudson, NH, USA). The water used in all experiments was treated by the Millipore Milli-Q purification system.

2.2. Synthesis of dopamine modified alginate

The dopamine modified alginate was synthesized by chemical reaction using EDC and NHS (Fig. 1A). 2 g of alginic acid sodium salt (about 10 mmol in terms of repeating unit) was dissolved in 100 ml distilled water at a concentration of 2% (w/v). And then PH value was adjusted to 5–6 with HCl. 5 mmol EDC and NHS were then added to the alginate solution. The reaction mixture was stirred at room temperature for 1 h to fully activate the carboxylic groups on alginate molecules. Then, dopamine hydrochloride (5 mmol) was added to the above solution and stirred for 12 h at room temperature under N_2 protection. The resulting solution was dialyzed against Milli-Q water over 24 h and its pH value was then adjusted to 8.5 using Tris-HCl. After overnight oxidation, the solution was dialyzed again for 12 h, and followed by freeze-drying. The product was stored at 4 °C before using.

2.3. Characterizations

The structure of the alginate-dopamine was confirmed by nuclear magnetic resonance (NMR). 10 mg alginic acid sodium salt and 10 mg alginate-dopamine were dissolved in 1 ml deuterium oxide 98% atom D (D_2O) separately. After complete dissolution, the solutions were transferred to NMR tubes for testing. The conjugation of dopamine to alginate backbone was determined by measuring the absorbance at 280 nm using a Uv-*vis* spectrophotometer, because the aromatic ring structure of conjugated catechol group exhibits absorption peak at this wavelength.

2.4. Cell culture

Bone marrow stem cells (BMSC) were obtained from 20 g C57BL female mice as follow: the bone marrow was aspirated from the mice femurs and washed with culture medium, and then centrifuged at 1000 rpm for 10 min. The cells were suspended and plated in 100 mm × 20 mm flasks (Greiner Bio-one, Monroe, NC, USA) in a basal medium composed of 1.0 g/l glucose dulbecco's modified eagle's medium (DMEM, Lonza, Walkersville, MD, USA) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 unit/ml

Table 1
The primers designed for each targeted genes.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
ALP	CTCAAAAGCTAACACCAATG	ATTTGTCCATCTCCAGCCG
BSP	CCACACTTTCCACACTCTCG	CGTCGCTTTCCTTCACTTTTG
COL-I	AACAGTCGCTTCACCTACAG	AATGTCCAAGGGAGCCAC
OPN	CTACGACCATGAGATTGGCAG	CATGTGGCTATAGGATCTGGG
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA