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Fabrication of stable galactosylated alginate microcapsules *via* covalent coupling onto hydroxyl groups for hepatocytes applications

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ABSTRACT

Galactose moieties are covalently coupled with sodium alginate to enhance liver-specific functions in microcapsules owing to the specific interaction between the galactose moieties and the asialoglycoprotein receptors (ASGPRs) of hepatocytes. In this study, galactosylated alginate (L-NH_2-OH-alginate) based microcapsules with desirable stability and a suitable 3D microenvironment are designed and fabricated for primary hepatocyte applications. The designed L-NH_2-OH-alginate is fabricated via the application of ethylenediamine grafted lactobionic acid (L-NH_2) onto the hydroxyl groups of sodium alginate so that the negatively charged carboxyl groups intact in L-NH_2-OH-alginate can effectively bond with Ca^{2+} to form a stable three-dimensional gel network; a subsequent reaction with polycations forms a stable membrane of microcapsules. As a result, L-NH_2-OH-alginate based microcapsules exhibit an excellent mechanical stability. Moreover, with a higher degree of substitution in L-NH_2-OH-alginate (DS 0.41), the hepatocytes entrapped in L-NH_2-OH-alginate microcapsules exhibit better viability and well-maintained liver-specific functions.

1. Introduction

Hepatic failure (HF), resulting from multiple causes, is a rare clinical syndrome responsible for extensive hepatocyte necrosis, severe liver functional damage and multiple organ failure (Ben Ari et al., 2012; Li et al., 2012). Thus, the restoration of hepatic function would not only improve the quality of life but also enhance the survival of patients with acute HF (Dixit et al., 1990). Whole organ liver transplantation has been applied for HF treatment in recent years due to its unparalleled advantage in recovering of hepatic function (Dixit et al., 1990). Unfortunately, the limited compatibility and sources of donor livers severely hampered its wide application in clinic (Dixit et al., 1990). To address this issue, biological artificial liver support system (BALSS), with the ability to secrete endogenous active substances and convert exogenous toxins, has increasingly become one of the most promising technologies for HF treatment (Bartlett & Newsome, 2015; Ding et al., 2003). Accordingly, many efforts have been made aiming to improve the viability and function of hepatocytes involved by the BALSS. However, their achievements are still far from creating functionally well-maintained hepatic tissue due to their limited capability of mimicking the native microenvironment of hepatocyte *in vitro*. This is important because a number of investigations have supported that hepatic tissue microenvironment including stromal cells, extracellular matrix (ECM) and even complicated cell-cell/cell-ECM interactions, play in dispensable role in regulating the phenotype and function of hepatocytes *in vivo*.

To better mimic the *in vivo* microenvironment, an enormous potential exists in bioengineering 3D culture system. In particular, microencapsulation technology has been demonstrated to be one of most powerful tools to reconstruct an appropriate 3D tissue-like microenvironment for hepatocytes. A series of studies by Renate Reinhardt's group have found that the function of primary hepatocytes could be well maintained when they were cultured within calcium alginate-polylysine (AP) microcapsules with collagen and matrigel incorporation (Dixit, Arthur, Reinhardt, & Gitnick, 1992). Our laboratory also has focused on the microcapsule-based 3D culture *in vitro* and *in vivo* in the past decades, and its competitive

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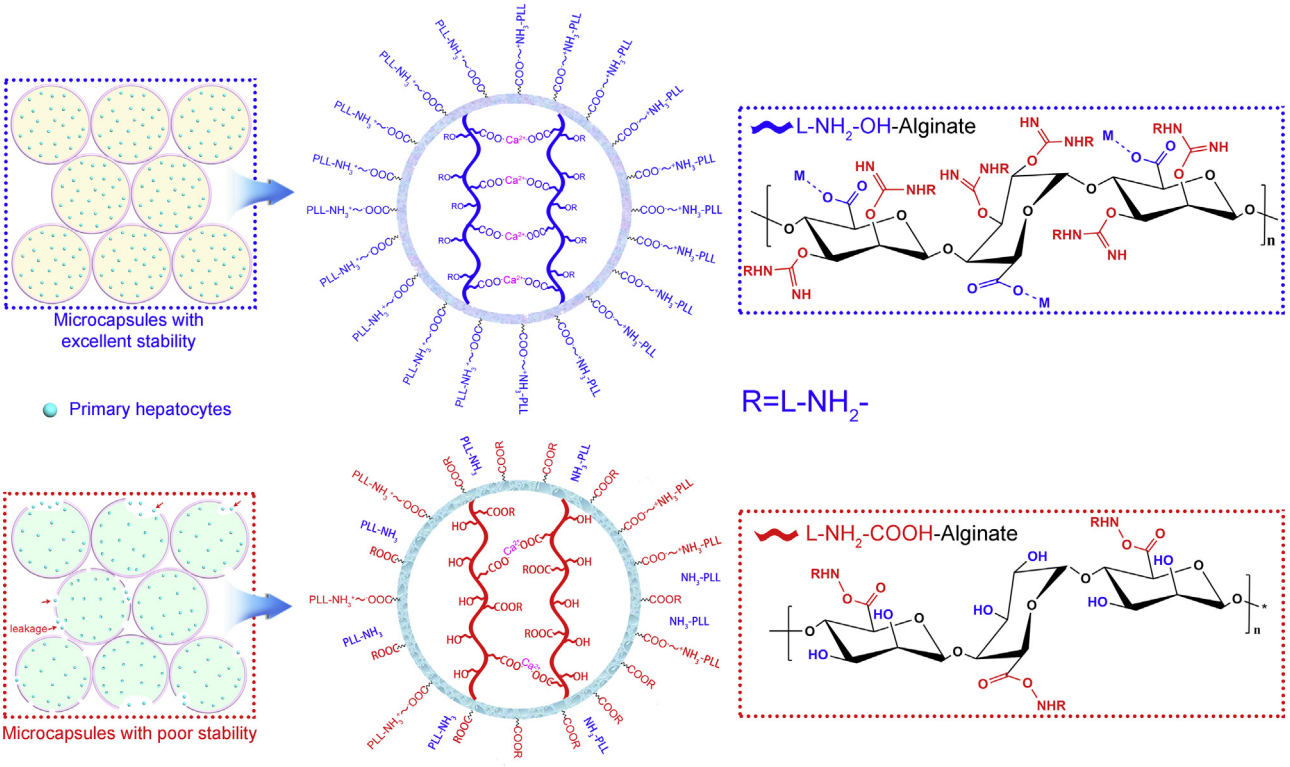

Scheme 1. Principle of designed microcapsules with excellent stability in primary hepatocyte applications.

advantages in supporting the phenotype and function of mammalian cells have been well documented. All these work suggests the potential application of microcapsule technique in biological artificial liver support system.

With respect to microcapsule-based hepatocyte culture *in vitro*, alginate, composed of β-D-mannuronic acid (M units) and α-L-guluronic acid (G units), has become the material most widely used to prepare biological microcapsules due to its perfect combination of biocompatibility, non-toxicity, non-immunogenicity and biodegradability (Bidarra, Barrias, & Granja, 2014; Lee & Mooney, 2012). Although microcapsules prepared from calcium alginate (G units of alginate chelate Ca^{2+} with the formation of tetrahedral coordination geometry, "egg-box" structure) and polycationic materials (such as chitosan and polylysine) displayed good biocompatibility and excellent mechanical stability (Thu et al., 1996), hepatocytes encapsulated by these microcapsules usually exhibit low viability and rapidly impaired liver function due to the lack of cell-cell and cell-matrix interactions (Yang, Goto, Ise, Cho, & Akaike, 2002). To address this issue, galactose groups have been recognized as an effective strategy by inducing and improving the function of hepatocytes because the abundant asialoglycoprotein receptors (ASGPRs) in hepatocytes have been shown to have the capability to recognize galactose as a specific ligand (Baenziger & Fiete, 1980; D'Souza & Devarajan, 2015).

Currently, both blending alginate with another macromolecular substance that contains galactose groups and covalently coupling galactose moieties with alginate are the most widely used methodologies for introducing galactose groups into microcapsules (Seo et al., 2005; Tian, Han, Tan, & You, 2014; Yang et al., 2002). However, the former usually results in leakage of the blended materials, further leading to poor stability of the microcapsules and hindering their application. In contrast, the latter had been demonstrated to be a more effective way to keep galactose in the microcapsules (Tian et al., 2014; Yang et al., 2002). But it should be noticed that modification of carboxyl groups in alginate would inevitably weaken the gelation reaction between Ca^{2+} and alginate. This could in turn lead to a remarkable reduction of the mechanical stability and integrity of the microcapsules (Sun, Chan, Quek, & Yu, 2004; Trinh & Schnabel, 1993; Yin et al., 2003). Moreover, the substitution degree of galactose on carboxyl groups in alginate cannot reach a much high value (higher DS will not lead to beads formation) due to the fact that the stability of microcapsules prepared from the material was extremely poor, which cannot improve the hepatocytes functionality sufficiently.

In the present study, a facile strategy for designing and fabricating a novel galactosylated alginate (L-NH_2-OH-alginate) was established by introducing ethylenediamine grafted lactobionic acid (L-NH_2) into the hydroxyl groups of sodium alginate (Scheme 1). Galactosylated alginate microcapsules (L-NH_2-OH-AP) were then fabricated and their reactivity and mechanical stability were further characterized. Moreover, the viability and liver functions of primary hepatocytes encapsulated by the L-NH_2-OH-alginate microcapsules were evaluated to elucidate their potential application in BALSS.

2. Materials and methods

2.1. Materials

Sodium alginate was purchased from the Chemical Reagent Corp (Qingdao, China). The Mw and G/M ratio of the alginate were 430 kDa and 35/65, respectively. Polylysine (PLL, Mw 15,000–30,000) was purchased from Sigma-Aldrich Chemical Co. (P7890MSDS, USA). Lactobionic acid was purchased from Energy Chemica (ShangHai,China). CNBr was purchased from the Aladdin Reagent Co. (ShangHai, China). All other reagents and solvents were of analytical grade and used without further purification.