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Locust bean gum as an alternative polymeric coating for embryonic stem cell culture

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ABSTRACT

Pluripotent embryonic stem cells (ESCs) have self-renewal capacity and the potential to differentiate into any cellular type depending on specific cues (pluripotency) and, therefore, have become a vibrant research area in the biomedical field. ESCs are usually cultured in gelatin or on top of a monolayer of feeder cells such as mitotically inactivated mouse embryonic fibroblasts (MEFsi). The latter is the gold standard support to maintain the ESCs in the pluripotent state. Examples of versatile, non-animal derived and inexpensive materials that are able to support pluripotent ESCs are limited. Therefore, our aim was to find a biomaterial able to support ESC growth in a pluripotent state avoiding laborious and time consuming parallel culture of MEFsi and as simple to handle as gelatin. Many of the new biomaterials used to develop stem cell microenvironments are using natural polymers adsorbed or covalently attached to the surface to improve the biocompatibility of synthetic polymers. Locust beam gum (LBG) is a natural, edible polymer, which has a wide range of potential applications in different fields, such as food and pharmaceutical industry, due to its biocompatibility, adhesiveness and thickening properties. The present work brings a natural system based on the use of LBG as a coating for ESC culture. Undifferentiated mouse ESCs were cultured on commercially available LBG to evaluate its potential in maintaining pluripotent ESCs. In terms of morphology, ESC colonies in LBG presented the regular dome shape with bright borders, similar to the colonies obtained in co-cultures with MEFsi and characteristic of pluripotent ESC colonies. In short-term cultures, ESC proliferation in LBG coating was similar to ESC cultured in gelatin and the cells maintained their viability. The activity of alkaline phosphatase and *Nanog*, *Sox2* and *Oct4* expression of mouse ESCs cultured in LBG were comparable or in some cases higher than in ESCs cultured in gelatin. An in vitro differentiation assay revealed that mouse ESCs cultured in LBG preserve their tri-lineage differentiation capacity. In conclusion, our data indicate that LBG coating promotes mouse ESC growth in an undifferentiated state demonstrating to be a viable, non-animal derived alternative to gelatin to support pluripotent mouse ESCs in culture.

1. Introduction

The large potential of ESCs for clinical applications, replacement therapies or tissue engineering is evidenced by their ability to self-renew and to differentiate into many cellular types [1]. General practices of ESC growth usually rely on their culture on a feeder layer of mitotically inactivated primary mouse embryonic fibroblast (MEFsi) to maintain them in a pluripotent state [2]. However, maintaining a parallel culture of MEFsi to the ESC culture is laborious and time-consuming, and certain procedures require additional steps for the separation of both cells. Therefore, many researchers culture ESCs in tissue culture polystyrene (TCPS) vessels coated with gelatin or Matrigel®, which are animal-derived protein solutions.

Gelatin, a translucent and colorless substance derived from collagen, is a cheap coating that has been vastly used to culture mouse ESC. As gelatin is an animal-derived material produced by partial hydrolysis of collagen extracted from the boiled bones, connective tissues and organs of animals [3], it poses problems for use in cell replacement therapies.

Nevertheless, in order to use ESCs in the aforementioned applications, they must be cultured in animal-free derived matrices. For that reason, several authors have been developing synthetic and natural alternative supports that could be used to maintain ESC pluripotency, enabling a subsequent application in basic stem cell biology and regenerative medicine [4–7]. Non-animal derived materials are less prone to induce problems for cell replacement therapies, namely immunogenic reactions,

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which is a clear advantage. Synthetic polymers are easily incorporated into a wide variety of materials, making their application very frequent. Nevertheless, replacing these polymers by natural counterparts is a parallel and cheaper approach, which further evidences high biocompatibility potential. Indeed, natural polymers are frequently used in nanocomposites or as coatings to improve the biocompatibility of metal implants, being highly recommended to make the bridge between synthetic devices and human tissue [8].

Polysaccharides are the most common polymers in nature, consisting of monosaccharides bound together through glycosidic bonds [9]. The most abundant polysaccharides include starch, cellulose, chitin, glycogen, galactomannans and carrageenans [9,10]. Among the galactomannans, locust bean gum (LBG) is obtained from the seeds of the carob tree (*Ceratonia siliqua*) where it normally acts as storage carbohydrate during germination. LBG has a mannose backbone with single side chain galactose units in a mannose/galactose ratio of approximately 4:1 (Fig. 1A). In general, LBG presents a molecular weight range of 50–1000 kDa [11]. LBG properties are generally unaffected by pH, salts, adsorption on solid surface or heat processing because it is non-ionic [12,13]. LBG was found to adsorb to solid surfaces to let more of its OH groups in contact with the surface through hydrogen bonding [13]. Furthermore, studies on textural properties of LBG showed that it presents high values of hardness, adhesiveness, springiness, chewiness and gumminess [14]. Therefore, LBG is commercially available and is widely applied in different areas, from food to pharmaceutical industry, due to its gelling and thickening properties [11]. Additionally, it is also used as stabilizer and emulsifier.

LBG is widely used in drug delivery systems [15], in biopharmaceutical applications [11] and to culture hepatocytes [16,17]. However its potential use in ESC culture remains unexplored. In this work a new application of LBG is described. The development of an LBG coating and its application in ESC culture as a support for undifferentiated and pluripotent mouse ESC growth is reported.

2. Materials and methods

2.1. Materials

Cell culture was performed in 6-well tissue culture polystyrene (TCPS) plates purchased from TPP (Switzerland). The process of polystyrene treatment generates highly energetic oxygen ions, which graft onto polystyrene chains on the surface so this becomes hydrophilic and negatively charged [18]. Gelatin type B, derived from lime-cured tissue bovine skin and tissue culture grade, was purchased from Sigma (Switzerland), has an isoelectric point of 4.7–5.2 [supplier data] and, at a concentration of 0.1%, has a pH = 7. LBG powder was a kind gift from Roeper (Germany). LBG from Roeper originates from Spain, Italy and Turkey, and is commercialized as a white to yellowish-white colored powder containing galactomannan (75% minimum), water (14% maximum), protein (7% maximum) and ash (1.2% maximum). LBG is a galactomannan and, as many polysaccharides, is a neutral material [11]. MEFsi were prepared from C57Bl/6 mice obtained from the animal facility of the University of Algarve, according to the literature [19]. MEFsi is a coating composed of cells, which membrane is negatively charged [20,21]. E14GPF8 mouse ESCs were provided by Dr Tristan Rodriguez (Imperial College London). Unless specified, cell culture medium components and reagents were purchased from Gibco (Life Technologies).

2.2. Characterization of natural polymeric coatings by scanning electron microscopy (SEM)

LBG coating was characterized using a field emission scanning electron microscope (FESEM; FESEM Ultra Plus, Zeiss, Germany) at 3 kV of voltage, which is equipped with a secondary electron (SE) detector. In brief, solutions of 0.1% (w/v) of both LBG and gelatin were incubated in TCPS coverslips overnight (o.n.), the excess of solution

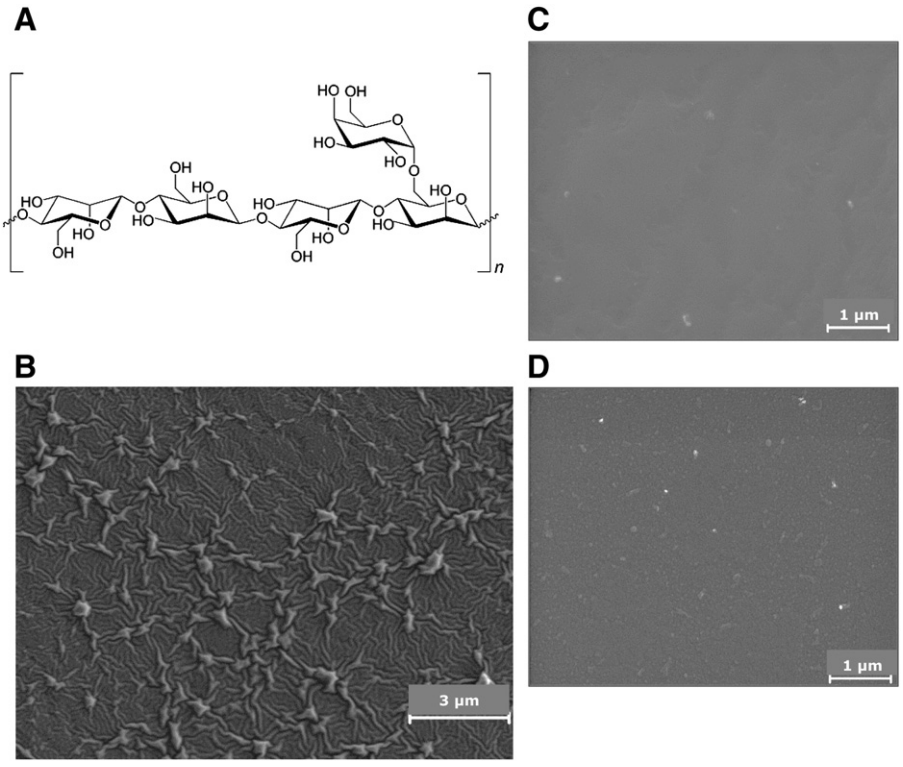

Fig. 1. Chemical and morphological structure of LBG. **A**. Chemical structure of locust bean gum showing a linear polysaccharide (1-4)-β-linked backbone of mannose units with single (1-6)-α-D-galactose units attached. Adapted from [11]. SEM morphology of LBG (**B**) and 0.1% gelatin coatings (**C**) and TCPS (**D**). The LBG polymeric film forms a striated and rough mesh.