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Research Paper

Rheological characterization of a gel produced using human blood plasma and alginate mixtures

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ABSTRACT

Human blood plasma is a material used to generate tissue equivalents due to presence of fibrinogen. However, gels formed using human blood plasma has weak mechanical properties. In this study, different mixtures of sodium alginate and blood plasma were performed and evaluated. By determining ζ potential can be established the stability of the plasma–alginate mixture and by dynamic rheology can determine the most suitable parameters for the gelation of the above mixtures, when calcium chloride is used as a crosslinker. Experimental results evidence an increment in ζ potential at alginate concentrations of 0.8% and 1.6% with a resulting pseudoplastic behavior of evaluated mixtures, which described the homogenization of the mixture. On the other hand, mixtures were gelled by using aspersion of calcium chloride and characterized by dynamic rheology. Solid behavior is dominant in all range of frequency sweep test between 0.1 Hz and 100 Hz. Finally, the ultimate tensile strength of a gel reach 6.36938 ± 0.24320 kPa, which is enough for manual handling of the gel. Between the tasks of the gel would be used for cell entrapment, for controlled release of drugs or in the manufacture of wound dressings.

1. Introduction

Although many materials have been evaluated and used in generation of temporary scaffolds for cells growth in tissue engineering (Yoon and Fischer, 2007; Pachence et al., 2007; Warren et al, 2004), research community prefer the use of biocompatible and biodegradable materials (Falke and Atala, 2000). These kind of materials enhance cell adhesion and signal capabilities (Sukmana, 2012).

Natural polymers provide these properties mentioned above. They can be divided in two major groups: proteins such as collagen, gelatin, albumin, fibrinogen, and polysaccharides like chitosan, hyaluronic acid, alginate, cellulose and dextran. The mixture of polysaccharides and proteins for generating tissue scaffolds gives a promising combination of good mechanical strength provided by polysaccharides and the cell adhesion capabilities of proteins.

Particularly, Fibrinogen has gained status in tissue engineering due to its ease of acquisition and its ability to generate diverse and different scaffolds. This material can be obtained either, from a patient's own blood plasma, blood bank plasma, or from commercial sources (e.g., Tissel®, Baxter Laboratories).

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Plasma proteins can be separated into three fractions: albumin (approximately 42%), globulins (56%), and fibrinogen (1%); other proteins, such as fibronectin, lipoproteins, and others represent less than 1% of the total protein content of plasma (Moure et al., 2003; Schaller et al., 2008).

Under normal conditions, fibrinogen is present in blood plasma as a zymogen, but in case of a wound, fibrinogen is activated through a serie of reactions known as the "coagulation cascade". During these reactions, multiple enzymes and substrates are involved and lead to a formation of fibrin fibers which, along with platelets, coagulates the wound (Eyrich et al., 2007). Also, it is important to mention that some studies (Meana et al., 1998; Arvelo et al., 2004; Llames et al., 2004; Zhao et al., 2008) do not perform fibrinogen purification, instead of that, they use blood plasma directly to exploit its native fibrinogen content and other proteins presented, giving a clinical advantage that reduces significantly the cost of tissue equivalent generation. However, due to their poor mechanical properties, the gels are not able to handle by medical personal. So, in order to enhance gel mechanical properties, one alternative is mix them with calcium alginate (Bhakta et al., 2009; Shikanov et al., 2009; Zhou and Xu, 2011; Ma et al., 2012).

On the other hand, alginate is a polysaccharide water-soluble polymer derived from algae (*Macrocystis pyryfera*, *Laminaria hyyperborea*, *Laminaria digitata*, and *Ascophyllum nodosum*) or from recombinant microorganisms (*Azotobacter vinelandii*). Alginate is a polyanion composed by two monomeric units: β-D-mannuronate (*M* units) and α-L-guluronate (*G* units) and linked by β (1–4) bonds (Yoon and Fischer, 2007; Brandl et al., 2007; Eiselt et al., 2000; Coviello et al., 2007; Shapiro and Cohen, 1997). Due to its polyelectrolytic nature, alginate is cross-linked when it is exposed to divalent ions, such as calcium or barium, in order to form a polymer network known as the "egg box" model (Grant et al., 1973; Brandl et al., 2007).

Alginate is preferred in tissue engineering for their ability to gel formation by ion displacement at 37 °C in isotonic solutions, is biocompatible and does not interfere with the cellular function of the immobilized cells (Mazzitelli et al., 2011). Since their hydrophilic behavior, alginate does not provide good performance of cell adhesion (Yoon and Fischer, 2007; Pokrywcznska et al., 2008). In order to improve cell adhesion, alginate usually is mixed with protein or peptide sequences in a way such that cells can be able to recognize the proteins through existing receptors in the cell membranes (Brandl et al, 2007; Mazzitelli et al., 2011). One of these proteins mixed with alginate for scaffold generation in tissue engineering is the fibrogen (Bhakta et al, 2009; Shikanov et al, 2009).

In order to obtain a suitable material for tissue engineering purposes, human blood plasma and sodium alginate were mixed and subjected to gelation by sprayed calcium chloride. The gel obtained had workability and had the potential to be used in applications such as scaffold for trapping cells, for controlled drug release or as a wound dressing.

2. Materials and methods

2.1. Reactives

Sodium alginate (Ref: A2033, 61% manuronic acid (M), 39% guluronic acid (G); ratio *M/G* 1.56; MW: 80.000–120.000) was purchased to Sigma-Aldrich Co (Saint Louis, MO, USA); calcium chloride (Ref: 433381) and sodium chloride (Ref: 479687) were purchased to Carlo Erba (Strada Rivoltana, Italy).

2.2. Blood plasma procurement

Human blood plasma was obtained from the blood bank at the District Health in Bogotá (Colombia). Type O+ plasma was used to perform all predetermined assays in this research study; plasma is not used for human patients for its high lipemic content. To validate all assays, 30 units of plasma (250 mL) were mixed under sterile conditions in a laminar flow hood after thawing and warming to 37 °C with a thermostat-controlled water bath (Cole-Parmer model 1266-02). To eliminate fibrinogen degraded during storage, the mix was centrifuged at 7100 × *g* for 20 min (Sorvall) at ambient temperature using 500 mL flasks (Nalgene, Rochester, USA). The supernatant was filtered using a Millipore filtration cartridge with an 8 μm pore size for eliminating lipemic material. Once the components were separated, the plasma was transferred into 500 mL dark amber glass containers (Schott, Mainz, Germany) and stored at −30 °C. Samples were thawed as needed for experimentation.

2.3. Determination of the ζ potential

ζ Potential was used for establishing the stability of mixtures in various proportions of alginate, to avoided precipitation in gel formation. Alginate/plasma mixtures were prepared to the following alginate concentrations: 0.2%, 0.4%, 0.8% and 1.6% (w/v) from human plasma and sodium alginate solution at 3% (w/v) in water. Then ζ Potential was determinated using an equipment ZETAMETER 3+ (Stauton, USA). The mixture was brought to a conductivity of 20 mS/cm with sodium chloride at ambient temperature using a conductimeter Oaklon 510; the last procedure was development for adjusting the ionic force to the same value in all assays.

2.4. Viscometry of blood plasma and sodium alginate

Viscometry assays were performed on human blood plasma, diverse sodium alginate solutions (0.2%, 0.4%, 0.8%, and 1.6% (w/v)), and sodium alginate/human blood plasma mixtures in the same sodium alginate proportions stated above. These assays were conducted with BOHLIN CVOR-200 Rheometer (Malvern Instruments Ltd, Worcestershire, UK) using a cone-and-plate geometry arrangement with an angle 2° and 40 mm of diameter. Assays were performed with an increasing ramp of shear rates between 0.1 s^{-1} and 150 s^{-1} for 100 s, remaining at 150 s^{-1} for 120 s, and finally descending from 150 s^{-1} to 0.1 s^{-1} for 100 s. All assays were performed in triplicate at a constant temperature of 37 °C.

2.5. Fabrication of blood plasma and alginate gels

Mixtures of plasma/alginate, 0.8% and 1.6% (w/v) in alginate, were distributed on a metallic support for producing a gel with height of 1 mm. Hollow metallic support of rectangular form was used for gellification; dimentions of support were 20 cm (length) × 20 cm (height) and 1 mm (height); on this