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Short communication

Albumin removal from human fibrinogen preparations for manufacturing human fibrin-based biomaterials

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Abstract

Commercially available two component human fibrin sealants are commonly used to manufacture human fibrin-based biomaterials. However, this method is costly and allows little room for further tuning of the biomaterial. Human fibrinogen solutions offer a more cost-effective and versatile alternative to manufacture human fibrin-based biomaterials. Yet, human fibrinogen is highly unstable and contains certain impurities like human albumin. Within the context of biomaterials and tissue engineering we offer a simple yet novel solution based on classical biochemical techniques to significantly reduce albumin in human fibrinogen solutions. This method can be used for various tissue engineering and biomedical applications as an initial step in the manufacturing of human fibrin-based biomaterials to optimise their regenerative application.

Keywords: Human fibrinogen; Human fibrin; Dialysis; Albumin; Biomaterials.

1. Introduction

Fibrin, an insoluble polymeric protein, is produced in response to bleeding during the last step of the blood coagulation cascade and is arranged in long fibrous chains [1]. The precursor of fibrin is fibrinogen, a soluble protein produced in the liver and found in blood plasma [2]. Tissue damage results in the cleavage of fibrinogen by thrombin and its assembly to form fibrin. Fibrin is known to play a pivotal role in wound healing, making it an ideal biomaterial choice for tissue engineering applications, aiming at restoring tissue structure and function [3,4].

Due to its poor mechanical properties and high biodegradability, fibrin, naturally a gel biomaterial, is often used in combination with other materials and/or chemically modified, i.e. cross-linking, to tune and control its physical properties. Thus, fibrin-based biomaterials are biocompatible, biodegradable, have high affinity towards biological surfaces, enhance cell attachment, tissue repair and support angiogenesis [1,4]. They also serve as suitable templates for controlled release of growth factors [5–7]. In addition, they have been used for stem cell delivery into the injury site, thus acting as cell carriers [8]. Due to its versatile nature, fibrin-based biomaterials have been tested as potential scaffolds to regenerate bone, cardiac tissue, cartilage, liver, nervous tissue, ocular tissue, skin, adipose tissue, tendons, and ligaments [9].

Fibrin requires the use of fibrinogen and thrombin as the starting materials for its preparation [10]. Commonly, commercially available two component human fibrin sealants are used to manufacture human fibrin-based biomaterials. However, this method is costly and allows little room for further modifications. Human fibrinogen solutions offer a more cost-effective and versatile alternative to manufacture human fibrin-based

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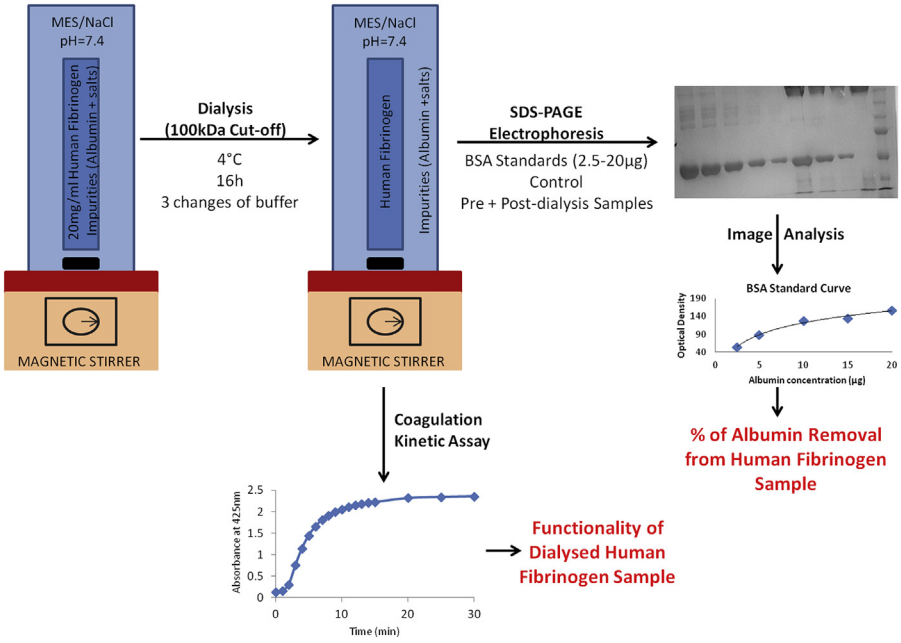

Fig. 1. Graphical summary of the method proposed in this paper for removal of albumin from human fibrinogen preparations for manufacturing human fibrin-based biomaterials.

biomaterials. Yet, human fibrinogen is highly unstable and contains certain impurities like human albumin and salts (i.e. L-arginine hydrochloride, sodium chloride, sodium citrate) to prevent its degradation [11,12]. Albumin is the most abundant protein in plasma and is structurally very stable due to the presence of 17 disulphide bonds. This allows albumin to remain stable under different conditions. However, its hydrophobic domains increase the hydrophobicity of the fibrin biomaterial (cells find it difficult to attach to hydrophobic surfaces) thereby reducing the applications for the biomaterial [13,14]. Most importantly albumin is also known to have an anti-coagulation effect on fibrinogen [14,15]. Due to the above mentioned reasons, albumin can have an inhibitory effect on fibrin activity during tissue regeneration and therefore its levels should be reduced from human fibrinogen solutions. Dialysis is commonly used to remove the salts present in protein solutions but albumin usually remains a concern due to its larger molecular weight. Hence, there is a need for a cost-effective and efficient method for removal of albumin from human fibrinogen solutions.

The aim of this work was to remove or significantly reduce the concentration of albumin present in human fibrinogen solutions using simple, classical biochemical techniques as an initial step in the manufacturing of human fibrin-based biomaterials. For this purpose, human fibrinogen was dialysed in dialysis tubing with a large molecular weight cut-off (100 kDa), which allowed albumin and other salts to pass through, leaving the fibrinogen in the tube. Protein samples (pre and post dialysis) were analysed using SDS-PAGE electrophoresis by differentiating between the molecular weight of the two proteins: human albumin has a molecular weight of approximately 67 kDa while human fibrinogen has an approximate molecular weight of 340 kDa. Finally, a simple and rapid coagulation kinetic assay was performed on the dialysed human fibrinogen to assess its functionality. Fig. 1 summarises the different steps of the method described in this paper. The ultimate goal of this work is to establish a simple, cost-effective and robust method for removing albumin from human fibrinogen solutions that can be universally used as a previous step in the manufacturing of human fibrin-based biomaterials intended for tissue repair.

2. Materials and methods

2.1. Human fibrinogen solutions

Human fibrinogen from Bio Products Laboratory Ltd. (BPL, UK) was used as a control as it has a purity of approximately 100% and fibrinogen from CSL Behring Ltd. (RiaSTAP®, CSL Behring Ltd., UK), with an approximate purity of 65–75 %, was used as the test product. The powders (1 g of fibrinogen + X g of albumin + Y g of salts) were reconstituted as per manufacturers' instructions, in sterile deionised water (50 ml). Samples from the diluted CSL protein solution (20 mg/ml of fibrinogen + unknown concentration of albumin) were collected and used as pre-dialysis samples.

2.2. Dialysis

The fibrinogen solutions were transferred into separate 100 kDa cut-off visking tubing (Spectra/Por® dialysis membrane, flat nominal width = 16 mm, diameter = 10 mm, Spectrum Laboratories Inc., US), sealed using dialysis clips and dialysed against 2-ethanesulfonic acid (MES)/NaCl buffer pH 7.4 (150 mM NaCl + 25 mM MES), with three changes over