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Alginate nanobeads interspersed fibrin network as *in situ* forming hydrogel for soft tissue engineering

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ABSTRACT

Hydrogels are a class of materials that has the property of injectability and *in situ* gel formation. This property of hydrogels is manipulated in this study to develop a biomimetic bioresorbable injectable system of alginate nanobeads interspersed in fibrin network. Alginate nanobeads developed by calcium cross-linking yielded a size of 200–500 nm. The alginate nanobeads fibrin hydrogel was formed using dual syringe apparatus. Characterization of the *in situ* injectable hydrogel was done by SEM, FTIR and Rheometer. The developed hydrogel showed mechanical strength of 19 kPa which provides the suitable compliance for soft tissue engineering. Cytocompatibility studies using human umbilical cord blood derived mesenchymal stem cells showed good attachment, proliferation and infiltration within the hydrogel similar to fibrin gel. The developed *in situ* forming hydrogel could be a suitable delivery carrier of stem cells for soft tissue regeneration.

1. Introduction

Biomaterials derived from natural sources are advantageous because of their inherent property of being recognized by the cells, which includes the presence of cell binding receptors, induction of growth factor and its binding sites, proteolytic and remodeling properties triggered by cell binding etc [1,2]. On the other hand, most synthetic polymers have the advantage of being inert, high mechanical integrity and can be easily processed unlike natural polymers. Optimizing the properties of natural polymers to provide the required mechanical strength and degradation rate to match up with the regeneration rate of tissues would be beneficial.

Injectable hydrogel provide a suitable platform for the delivery of cells, drugs, proteins etc to the site *via* a minimally invasive technique in addition to providing a cross-linked swollen network of biopolymer with soft compliance, mimicking soft tissues. Hydrogels also possess the advantage of having a highly permeable structure aiding in the efficient exchange of nutrients and oxygen [3]. Injectable *in situ* forming hydrogels acts as a suitable depot for the effective delivery of cells to the defect area. Soft tissue reconstruction is often a priority when it comes to damage or removal of soft tissue. Injectable *in situ* hydrogels is minimally invasive, can completely fill the defect site irrespective of its irregularity and is patient compliant that it overcomes the discomfort occurred during surgical procedures. The use of synthetic materials to augment soft tissue regeneration presents some limitations for host tissue integration [4] and thereby projects the need for blending or chemical modifications of the same. Natural polymers being biocompatible and with mechanical strength matching the tissue elasticity would be a suitable alternative. Fibrinogen, the monomer of fibrin, is composed of two sets of three polypeptide chains named Aα, Bβ, and γ, which are joined together by six disulfide bridges [5]. Fibrin is formed after thrombin-mediated cleavage of fibrinopeptide A from the Aα chains and fibrinopeptide B from the Bβ chains. This generates the fibrin monomer that has a great tendency to self-associate and form insoluble fibrin [6]. Fibrin has been widely used in clinics as sealants. A number of allogeneic fibrin sealants such as Tisseel®, Evicel™, and Crosseal™ have been approved by the Food and Drug Administration (FDA) for clinical use as hemostatic agents [7]. Unlike a synthetic hydrogel, fibrin is not just a passive cell delivery matrix, but it binds specifically many growth factors as well as clot components, such as fibronectin, hyaluronic acid and von Willebrand factor [8]. Fibrin has two pairs of RGD sites and a pair of AGDV sites through which it can interact with integrins and has several sites that interact with

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the leucocyte integrin αmβ2 [9]. This bioactivity makes fibrin an attractive matrix for stem cell differentiation and tissue engineering. The softness and large compliance of fibrin gel make it effective for soft tissues. By modulating the mechanical and chemical properties of a fibrin-based matrix stem cell differentiation and tissue regeneration can be effectively carried out. In order to improve the low mechanical stiffness for some tissue engineering applications, fibrin hydrogel can be combined with other scaffold materials to obtain constructs with desired mechanical strength [7,10].

Alginate is a widely studied polysaccharide because of its structural resemblance to the extracellular matrix glycosaminoglycans [11]. It is a natural polymer extracted from brown algae. Water soluble alginate gelates when reacted with di- or tri-valent counter ions. Alginate gels are extensively studied for tissue engineering applications as a cell encapsulation material as well as an injectable 3D matrix for *in vivo* cell delivery. Several reports demonstrated that calcium alginate gel exhibits poor bioresorbability, biodegradation and cell adhesion except its easy preparation. As a biomaterial alginate is used because of its biocompatibility and hydrophilic nature and also due to its easy injectability [12,13]. Alginate cannot be broken down enzymatically and thus has a regulated degradation. Concerns have also been expressed on its immunogenicity and low cell adhesiveness [3]. Hwang et al. developed alginate particle embedded fibrin injectable hydrogel which showed an improved in growth of soft tissue *in vivo* [4]. They had developed alginate particles of higher weight percentage yielding non homogeneous micron sized particles [4]. Although the developed hydrogel showed enhanced neo tissue formation *in vivo* the higher percentage of alginate would pose issues of degradation. Microgels developed using alginate and fibrin also showed enhanced cell retention and viability and showed dramatic increase in retention *in vivo* [14].

The purpose of our study was to develop an injectable *in situ* forming alginate fibrin hydrogel, which could mimic the native tissue elasticity as well as feature enhanced cell material interaction with the minimum use of components. So in this work we developed nanobeads of alginate with the aim of reducing the total alginate content in the construct without compromising the material integrity. In addition the formation of nanobeads would open up other prospect like growth factor or cell delivery. These nanobeads would be incorporated into fibrin in the form of an *in situ* developing hydrogel. The higher surface area to volume ratio for nanobeads would help it to interact with fibrin matrix. The developed alginate nanobeads interspersed fibrin hydrogel was characterized using SEM, FTIR, rheological analysis and its efficiency to depot cells was evaluated *in vitro* by live dead assay, DNA quantification and fluorescence imaging.

2. Materials and methods

2.1. Materials

Alginic acid-Sodium salt was purchased from Aldrich Chemicals, India. Calcium Chloride Dihydrate Extra Pure and Thrombin (bovine plasma) were purchased from Merck, India. Primary antibodies used were purchased from Millipore, India and stored under −20 °C. Secondary antibody and actin specific stain were purchased from Molecular Probes, India. Fibrinogen required was isolated from human blood plasma pooled from healthy volunteers after their consent by cryoprecipitation method [15]. Duplojet Applicator was purchased from BD Sciences, India.

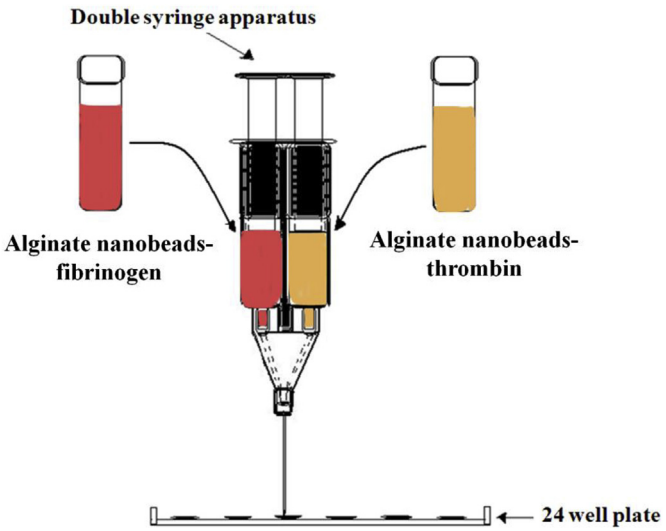

Fig. 1. Schematic diagram showing the method of gel synthesis.

2.2. Preparation of alginate nanobeads

Alginate nanobeads were prepared by spraying 1% w/v alginate solution into a bath containing 1 M $CaCl_2$ using a custom made atomiser. The nanobeads were then filtered out. The size range of the particles was obtained by Scanning Electron Microscopy (JEOLJSM-6490LA).

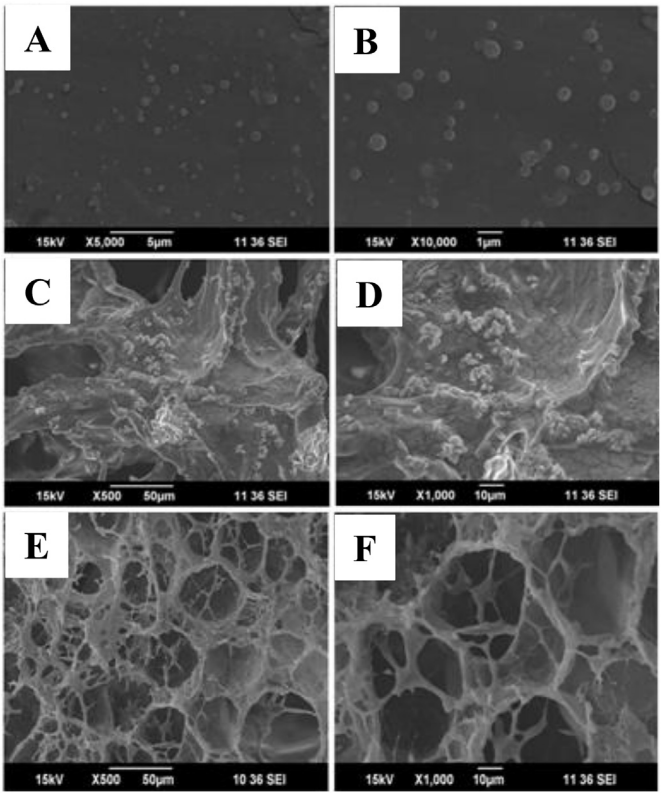

Fig. 2. SEM images of alginate nanobeads (A, B); alginate nanobeads fibrin composite (C, D); fibrin (E, F).

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