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Fabrication and characterization of novel nano-biocomposite scaffold of chitosan–gelatin–alginate–hydroxyapatite for bone tissue engineering

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ABSTRACT

A novel nano-biocomposite scaffold was fabricated in bead form by applying simple foaming method, using a combination of natural polymers–chitosan, gelatin, alginate and a bioceramic–nano-hydroxyapatite (nHAp). This approach of combining nHAp with natural polymers to fabricate the composite scaffold, can provide good mechanical strength and biological property mimicking natural bone. Environmental scanning electron microscopy (ESEM) images of the nano-biocomposite scaffold revealed the presence of interconnected pores, mostly spread over the whole surface of the scaffold. The nHAp particulates have covered the surface of the composite matrix and made the surface of the scaffold rougher. The scaffold has a porosity of 82% with a mean pore size of 112 ± 19.0 μm. Swelling and degradation studies of the scaffold showed that the scaffold possesses excellent properties of hydrophilicity and biodegradability. Short term mechanical testing of the scaffold does not reveal any rupturing after agitation under physiological conditions, which is an indicative of good mechanical stability of the scaffold. In vitro cell culture studies by seeding osteoblast cells over the composite scaffold showed good cell viability, proliferation rate, adhesion and maintenance of osteoblastic phenotype as indicated by MTT assay, ESEM of cell–scaffold construct, histological staining and gene expression studies, respectively. Thus, it could be stated that the nano-biocomposite scaffold of chitosan–gelatin–alginate–nHAp has the paramount importance for applications in bone tissue-engineering in future regenerative therapies.

1. Introduction

Globally, approximately 2.2 million bone-grafting procedures are performed every year on the pelvis, spine and other body extremities in attempts to repair or reconstruct the bone [1]. To promote bone growth, surgeons often use bone grafts or substitute materials [2]. Research on bone graft has begun to focus on bone tissue engineering, which involves the combination of cells, scaffold and bioactive agents to engineer new functional tissues that can replace the damaged tissues [3].

Natural bone is a complex of inorganic–organic nanocomposite materials. The primary tissue of bone is relatively hard and mostly made of inorganic material calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] nanocrystallites, which provide rigidity to the bones. The organic part of bone matrix is mainly composed of Type I collagen, an elastic protein which improves fracture resistance and aids in cell growth, proliferation and differentiation [4,5]. Other organic components present in the bone tissue include glycosaminoglycans, osteocalcin, osteonectin, bone sialoprotein and osteopontin. The inorganic matrix of the bone has great strength but is brittle (can break itself), while the organic matrix (e.g., collagen fibers) is flexible and has relatively low strength: inorganic hydroxyapatite and organic collagen (and other materials) together form a matrix that is strong and flexible enough not to be brittle [6,7].

As bone extracellular matrix (ECM) comprises of a variety of components, a scaffold for bone regeneration, if fabricated using single material like nHAp or collagen, cannot provide essential cues for cellular growth. However, two or more materials in combination, if used for scaffold fabrication, might generate a synergistic effect to provide good mechanical strength to the scaffold as well as facilitate cell adhesion, proliferation and differentiation [8–11]. Nowadays, scientists have been focusing on fabricating scaffolds using multi-polymers (more than two polymers) to mimic the properties of ECM, which also consists of multi-polymers [8,12–19]. Here, in this study, we focus on fabricating a composite scaffold with a combination of multi-polymers along with hydroxyapatite, for bone tissue engineering applications.

A scaffold can be fabricated by various techniques, and there are some advantages as well as disadvantages associated with each

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technique [20,21]. Among the available techniques, the foaming method — generating polymer-foam upon agitation of polymer solution and thereby crosslinking/solidifying the polymer-foam, is one of the simplest and most economic techniques available for scaffold fabrication [8,12,22], but it was not given much attention for tissue engineering applications. That is why we want to explore the foaming method for fabricating multi-polymer composite scaffold. The foaming method applied here is explained in detail in the next section (Section 2).

To fabricate a composite scaffold we selected three polymers, chitosan, gelatin and alginate, and nHAp. The reason for choosing this polymer combination along with nHAp is discussed below.

Although there are many different protein or polysaccharide molecules used in scaffold preparation, chitosan, gelatin and alginate have gained much attention for scaffold fabrication because of their availability, easy handling and low cost [23]. Chitosan is a natural polymer comprising glucosamine and N-acetylglucosamine obtained by the deacetylation of chitin [24]. Since it is degraded by the enzymes of human body, producing non-toxic side products, it is widely used in tissue engineering constructs [25]. Moreover, chitosan has antimicrobial and hemostatic properties [26–28]. Chitosan is osteoconductive, which makes it suitable for engineering hard tissues, but its mechanical properties and biological activities need to be enhanced [29]. For better mechanical properties, chitosan can be modified by blending with other natural polymers like silk, alginate, gelatin or ceramics, such as, tricalcium phosphate and hydroxyapatite [30,31]. Gelatin is a protein derived from collagen and contains Arg-Gly-Asp (RGD) sequences found in ECM, therefore, it promotes initial cell attachment and increases cell spreading, even more than chitosan [32]. Alginate, a naturally occurring polysaccharide, is biocompatible, but it lacks specific cellular interactions, which limits its potential use for wider applications. On the other hand, alginate, in presence of multivalent cations, produces a mechanically strong scaffold [33].

The use of nHAp particles in scaffold fabrication can incorporate nanotopographic features that mimic the nanostructure of natural bone [3,5]. Besides this, nHAp particles can provide the scaffold good mechanical strength, which is a prerequisite of the scaffold to be applied for bone regeneration [6]. The presence of nHAp in the scaffold also has a strong influence on bone regeneration [23,24].

Thus, if chitosan, gelatin, alginate and nHAp are combined all together during scaffold fabrication then this combination would impart the scaffold higher mechanical strength and biological similarity to the natural bone. Therefore, the specific goal of the present study is to fabricate a chitosan–gelatin–alginate–nHAp composite scaffold by exploring the simplest foaming method using commercially available nHAp to (i) improve the mechanical properties of the composite scaffolds relative to chitosan–gelatin–alginate scaffolds and (ii) to evaluate the biological performance of the nHAp incorporated scaffold for bone tissue engineering applications. To the best of our knowledge, this polymer combination along with nHAp is novel and has not been used before for scaffold fabrication.

2. Materials and methods

2.1. Fabrication of nano-biocomposite scaffold

Fig. 1 explains the fabrication of nHAp–chitosan–gelatin–alginate composite scaffold by the foaming method without using any surfactant. Solutions of 2 wt% alginate (Acros Organics, New Jersey, U.S.A.) and 5 wt% gelatin (Merck Specialities Pvt. Ltd., Mumbai) were prepared in sterilized water, and mixed in the ratio of 1:1. Thereafter, $NaHCO_3$ (0.9%), a gas generating agent, was added to this mixture and was continuously stirred for 2 h whereby foam is generated. Next, 0.025% glutaraldehyde solution (SD Fine-Chem. Ltd., Mumbai, India), crosslinker for chitosan [34] and gelatin, was added to the alginate–gelatin–$NaHCO_3$ foam matrix with a volume ratio of 1:40, and allowed to react (or crosslink) with the mixture for 10 h under the condition of continuous agitation. On addition of glutaraldehyde to this polymer mixture (Step 2 in Fig. 1), aldehyde groups present in glutaraldehyde, were believed to crosslink with the amino groups of gelatin present in the mixture [8,35]. Because of the continuous agitation, foam was generated in a large volume. The polymer foam was extruded drop-wise into a solution

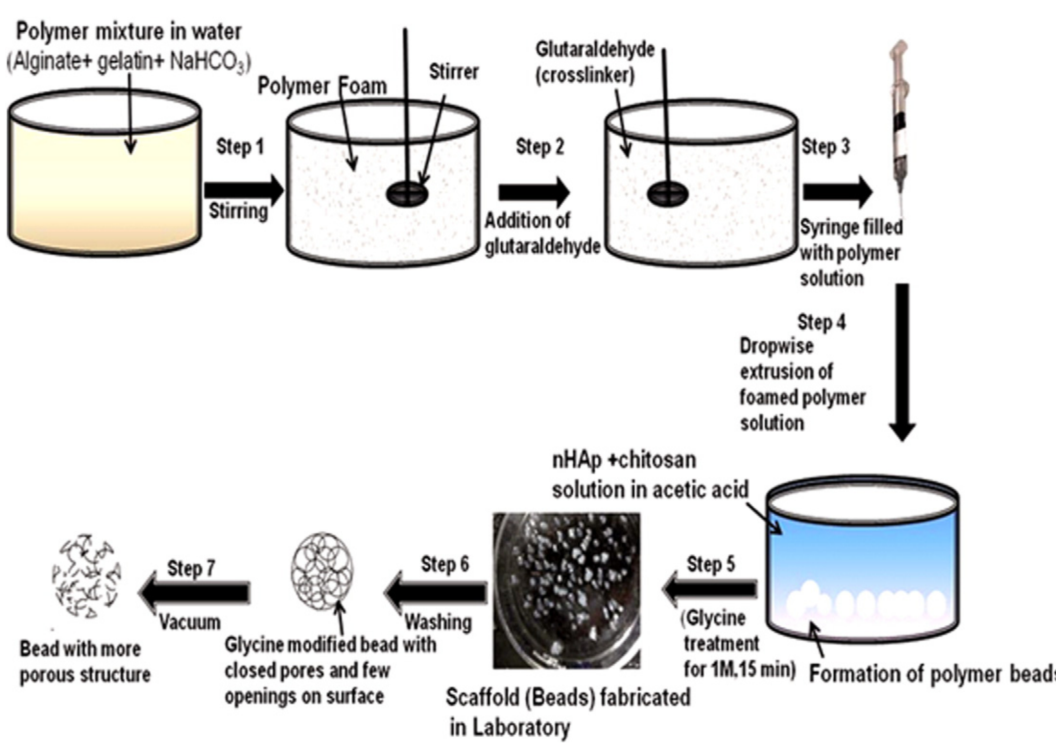

Fig. 1. Schematic representing fabrication of nHAp–chitosan–gelatin–alginate composite scaffold by foaming method.