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Development and characterization of a novel porous small intestine submucosa-hydroxyapatite scaffold for bone regeneration

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ABSTRACT

The fabricated small intestine submucosa (SIS) – hydroxyapatite (HAp) sponges can act as biomimetic scaffolds to be utilized in tissue engineering and regeneration. Here we developed SIS-HAp sponges and investigated their mechanical, physical and chemical characteristics using scanning electron microscopy, Fourier transformed infrared spectroscopy, uniaxial compression, porosity, and swelling testing techniques. The results demonstrated mechanical properties superior to comparable bone substitutes fabricated with similar methods. SIS-HAp scaffolds possess an interconnected macroporosity, similar to that of trabecular bone, hence presenting a novel biomaterial that may serve as a superior bone substitute and tissue scaffold.

1. Introduction

The bone matrix is the major structural connective tissue of the body, which consists of collagen (COL) and hydroxyapatite (HAp) in a complex and hierarchical structure. Although bones are known for their self-healing ability, factors such as aging, genetics and certain types of trauma are known to impair the ability of bone to retain its strength or heal. The global incidence of bone pathologies is rising, in part, due to increases in aging demographics [1,2]. In surgery, the gold standard of Bone Tissue Engineering (BTE) is the osseous autograft, which, aside from being histocompatible and osteoconductive, provides structural support and promotes the gradual replacement of old bone with new bone. However, autografts are limited by the availability of starting material, and encounter many challenges inherent to the implantation process, such as: prolonged surgical time for graft harvesting, donor site pain, inflammation, and high cost. Hence, research on BTE is important in addressing this worldwide problem. In addition to autografts, the field of BTE has proposed other bone repair techniques such as cellular therapy and a wide diversity of biomaterials. One of the best biomaterials include COL and HAp in the form of porous scaffolds. However, no biomimetic scaffold has all of the following properties simultaneously: high osteoinduction, affordability, and availability to physicians.

Recent studies have demonstrated that scaffolds based on Small Intestine Submucosa (SIS) [3], or HAp [4] can be degraded as new bone tissue grows [3]. SIS is a powerful, biocompatible, biodegradable and high elastic polymer that contains mainly COL type I and III (that constitute >90% of SIS), as well as small amounts of type IV and VI, which increase the structural integrity of bones [5]. Although SIS primarily consists of COL, it is a superior biomaterial due to the fact that SIS also contains extracellular matrix (ECM). The ECM components provide SIS an added advantage because they promote cell growth and survival due to endogenous growth factors and glycosaminoglycans (GAGs), such as fibronectin and hyaluronic acid [4].

SIS has been used in biomaterials to regenerate damaged soft tissues, such as blood vessels [6], bladder [7], diaphragm, dura mater, muscles, skin, ligaments, tendons and menisci [4]. Furthermore, SIS has been recently used as a regenerative matrix for bone grafts. SIS is beneficial in the guiding and attachment of host cells, forming new bone in a predefined shape. HAp, on other hand, is a bioceramic that has been widely investigated as a bone-like composite since HAp promotes osseous integration with significant formation of new bone. Furthermore, HAp porous scaffolds induce fast cell in-growth into the high-porosity regions, and withstand physiological and mechanical stress in the low-porosity regions when they are implanted [8]. Due to their natural bone composition, SIS-HAp composites are thought to become a bone graft more useful than traditional composites made from COL-HAp or only one of these components. Since the properties of SIS-HAp sponges have not been investigated, research on this composite is required, as well as studies on the effects of ceramic reinforcements on SIS sponges. Therefore, to assist in addressing this knowledge gap, the aim of this research is to develop experimental SIS-HAp sponges, and characterize their mechanical, chemical, physical, and morphological properties. In order to assess their properties, we use HAp - as a reinforcement - and *N*-(3-dimethylaminopropyl)-*N*′- ethylcarbodiimide hydrochloride

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(EDC) - as a crosslinking agent - to evaluate their potential use for bone tissue regeneration, and propose a novel scaffold as bone substitute.

2. Materials and methods

2.1. Materials

SIS was obtained from porcine small intestine of freshly slaughtered animals and processed in the Tissue Engineering Laboratory at Universidad de los Andes. HAp (nanopowder, <200 nm particle size), pepsin and EDC were supplied by the company Sigma-Aldrich.

2.2. Preparation of SIS

SIS was isolated via mechanical removal of the tunica serosa and tunica muscularis. Then, SIS was washed, dried, and pulverized using methods of the Tissue Engineering research group of the Biomedical Engineering Department at Universidad de los Andes.

2.3. Fabrication of SIS-HAp sponges

SIS-HAp sponges were developed by two protocols: EDC was used as the crosslinking agent in the first protocol only. A SIS solution (0.5% w/v) was used as the binder. SIS powder (particle size = 470 nm) was dissolved in acetic acid (3% v/v) and pepsin (0.1% w/v), as described elsewhere [9]. Then, the SIS blend was stirred for 48 h at physiological temperature (37 °C) as is suggested by Moon Suk Kim and Lee [10]. The SIS solution was carefully poured into 0.6 mL silicone molds - to form a cylindrical shape - and then freeze-dried. The mold size (base = 1 cm, height = 1 cm) was chosen to develop an economic scaffold fabrication technique and optimize the materials used for suspension performance. In order to achieve a HAp reinforcement of 1–2% (w / v), HAp nanopowder (<200 nm) was added to the SIS solution in the following proportions: 1:1, 1:2, and 1:4 SIS:HAp.

EDC crosslinked and non-crosslinked SIS scaffolds were fabricated as an experimental control. The crosslinked SIS sponges were immersed in EDC solution (50 mM) for 24 h. Next, crosslinked samples were washed in deionized water (DW) at room temperature (RT) to remove EDC. After 1 h, SIS sponges - in molds - were lyophilized for 24 h to produce the final construct, which corresponds to the control. Both protocols, with and without EDC, were performed to determine the effect of HAp reinforcement in SIS sponges. Specific concentrations were chosen in agreement with the SIS concentration used in the Tissue Engineering Laboratory at Universidad de los Andes. This concentration also allowed us to test HAp reinforcement in SIS scaffolds at proportions used previously in biomimetic constructs [11].

2.4. Characterization of SIS-HAp sponges

2.4.1. Scanning electron microscopy (SEM)

SIS-HAp scaffolds were examined using SEM (JEOL JSM-6490LV, 15 kV). Samples were treated with sputter-coated gold-palladium using Dentom Vacuum Desk IV metallizer. Structural features of scaffolds, such as the pore shape and diameter, were characterized using

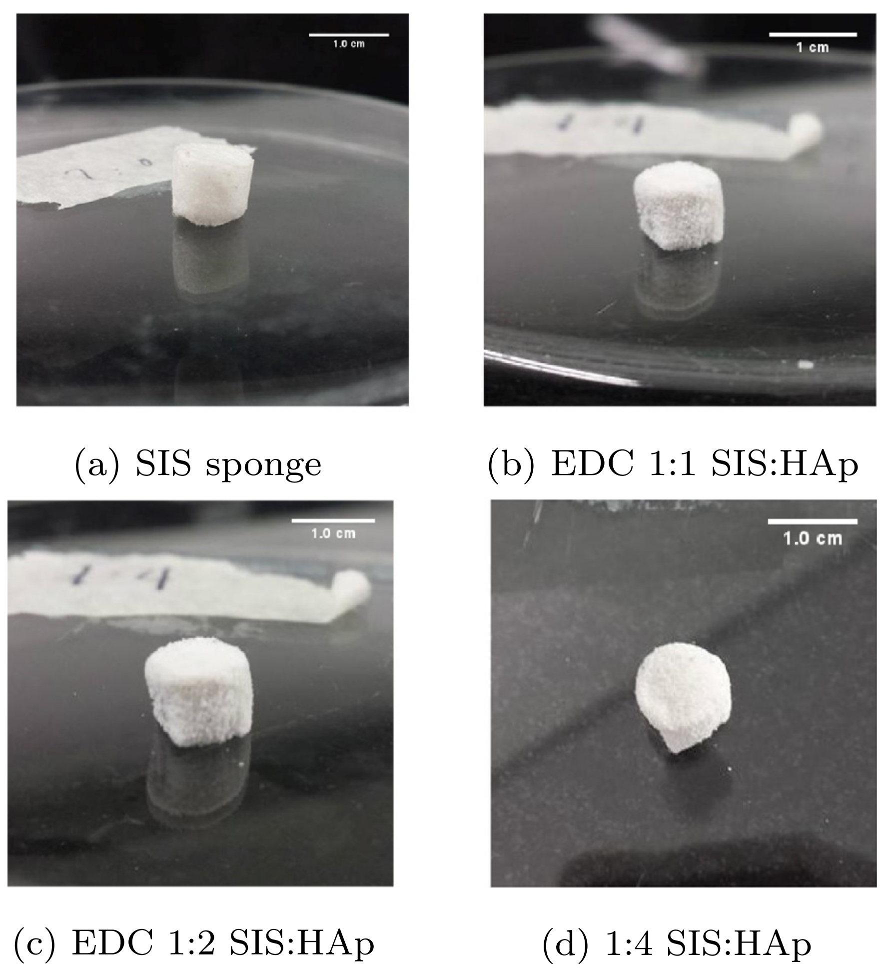

Fig. 1. Physical appearance of SIS:HAp samples. The SIS:HAp sponges exhibited an elastic property by touch. All sponges were white in color. Scaffolds became more opaque when HAp content increased. Samples were visually porous. Specifically, the 1:4 SIS:HAp sponges showed a greater tendency to crack than the other fabricated sponges.