International Journal of Biological Macromolecules

Volume 278, Part 4, October 2024, 135014

3D chitosan/hydroxyapatite scaffolds containing mesoporous SiO2-HA particles: A new step to healing bone defects

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https://doi.org/10.1016/j.ijbiomac.2024.135014

Abstract

Biocompatible scaffolds with high mechanical strengths that contain biodegradable components could boost bone regeneration compared with nondegradable bone repair materials. In this study, porous chitosan (CS)/hydroxyapatite (HA) scaffolds containing mesoporous SiO_2-HA particles were fabricated through the freeze-drying process. According to field emission scanning electron microscopy (FESEM) results, combining mesoporous SiO_2-HA particles in CS/HA scaffolds led to a uniform porous structure. It decreased pore sizes from 320 ± 1.1 μm to 145 ± 1.4 μm. Moreover, the compressive strength value of this scaffold was 25 ± 1.2 MPa. The *in-vitro* approaches exhibited good sarcoma osteogenic cell line (SAOS-2) adhesion, spreading, and proliferation, indicating that the scaffolds provided a suitable environment for cell cultivation. Also, *in-vivo* analyses in implanted defect sites of rats proved that the CS/HA/mesoporous SiO_2-HA scaffolds could promote bone regeneration *via* enhancing osteoconduction and meliorating the expression of osteogenesis gene to 19.31 (about 5-fold higher compared to the control group) by exposing them to the bone-like precursors. Further, this scaffold's new bone formation percentage was equal to 90 % after 21 days post-surgery. Therefore, incorporating mesoporous SiO_2-HA particles into CS/HA scaffolds can suggest a new future tissue engineering and regeneration strategy.

Introduction

Bones are one of the most prominent organs in the body and play a significant role in supporting and protecting tissues [1]. Millions of patients suffer from bone-related problems, aging-related generation growth, and pathological injuries all over the world [2,3]. Annually there are over than 20 million bone tissue losses, 5.5 million fractures, 1 million bone repair surgeries, and an estimated 2.2 million orthopedic procedures. This rate is predicted to increase by 13 % [4,5]. Bone grafting methods such as autogenous, allogenic, dynamic external fixation, and intramedullary pins have been the golden ways of treating different kinds of bone defects for several years [[6], [7], [8], [9]]. An estimated 500,000 to 600,000 bone grafting procedures are carried out by bone surgeons in the United States

alone each year [5]. Nevertheless, all these traditional methods have a lot of drawbacks including negative immune responses such as excessive inflammation, interference with healing, implant rejection, morbidity of the donor site, infection, and disease transmission [10]. So, the need for a second surgery is unavoidable. Considering the importance of bone, its related severe orthopedic challenges in the healthcare system, and the limited successful outcomes *via* current treatment strategies like autologous, the need for new approaches has become undeniable in bone tissue engineering [11]. Today, biodegradable scaffolds with biocompatible materials are considered promising bone tissue regeneration strategies. The favorable scaffolds for this aim should be able to imitate the natural process of bone regeneration and interact between three main ingredients *i.e.* cells, growth factors, and extracellular matrices [12,13]. In addition, the scaffolds should be osteoconductive, with adequate chemical stability, and sufficient mechanical strength [14]. In previous years, researchers and orthopedic surgeons have been attempting to find biomaterials with satisfactory qualities to fabricate high-featured scaffolds. Synthesis and natural polymers (*e.g.* collagen [15], gelatin [16], polycaprolactone [17], polylactic acid [18], *etc.*), and bioactive ceramics are common candidates. Among these biomaterials, chitosan ($(C_6H_{11}O_4N)_n$, CS) is a natural polymer with meritorious properties such as biodegradability, hydrophilicity, bioactivity, antimicrobial activity, and non-toxicity which make it possible to use in a wide range of biomedical applications [[19], [20], [21], [22]]. On the other hand, the poor mechanical properties, low porosity, and swelling ratio of pure chitosan induced investigators to blend it with another biomaterial [23,24]. Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA), a bioactive ceramic, is very similar to a natural human bone mineral in chemical and structural aspects. Its biocompatibility, bioactivity, and osteoconductivity properties make it appropriate to be used as an artificial bone filler for repairing bone defects and forming bone goals [[25], [26], [27], [28]]. Moreover, the release of Ca^{2+}, a well-known osteoinduction promoter, facilitates bone regeneration [29]. Even though it is expected that the combination of chitosan and hydroxyapatite will overcome both deficiencies and result in the fabrication of a better scaffold [30], there are some disadvantages such as low porosity volume, heterogeneous distribution of the ceramic phase in the polymer matrix, and low mechanical properties [31]. Consequently, adding a third compound to overcome these impediments and reach much better properties is inevitable. So, it could be a proper choice to incorporate mesoporous silica nanoparticles (MSNs) into CS/HA scaffolds due to their high specific surface area, pore volume, and chemical and thermal stability [31,32]. Also, studies showed that in mesoporous silica-based systems, particle size, shape, and surface chemistry have significant roles in biological performance [33]. Despite all these

advantages, because of the strong Si O skeleton in MSNs, they are unsuccessfully struggling with degradation in the human system [34]. In addition, they also suffer from a lack of bioactivity, which is a critical factor in bone tissue [35,36]. Thus, the amalgamation of MSNs with Ca^{2+} and PO_4^{3-}, bone-like precursors of HA, boosts the weaknesses of pure MSNs. Si^{4+} and HA can be released simultaneously, so, not only will MSNs be washed away from the body, but they will also favor bone formation by providing them with nutritious Si elements [37,38]. Varied methods are used to fabricate scaffolds, such as gel-casting, space holder, 3D printing, freeze-drying, electrospinning, *etc.* [39]. The economic benefits and

simplicity of the use of the freeze-drying method attract a vast number of experimenters. Porous scaffolds with controllable shapes can be achievabled using this technique [40].

Notwithstanding previous studies on bone regeneration, they have some disadvantages, like the longer healing process. A study by Cho et al. [41] showed remarkable bone regeneration in scaffolds with the combination of hydroxyapatite and polycaprolactone after eight weeks post-surgery. In another study by Oryan et al. [2], the 3D polylactic acid/polycaprolactone/hydroxyapatite scaffolds` bone regeneration process was completed after 80 days of surgery. Jin et al. [42] fabricated porous hydroxyapatite/chitosan/alginate scaffolds, assessed their bone regeneration process, and observed newly formed bone tissue after eight weeks in the site implanted with the scaffold. Although the biological and biocompatibility evolution of sarcoma osteogenic cell lines in the CS/HA/mesoporous SiO_2-HA scaffolds has mainly been explored using *in-vitro* analysis in our previous investigation [43], to get more credible evidence of the biocompatibility and potential application of these scaffolds, it is essential to address their bio-reactivities *in-vivo* in non-weight-bearing bone sites to avoid any intentionally effective parameters. To the best of our knowledge, no research has been published in the literature outlining the effect of mesoporous SiO_2-HA particles in *in-vivo* conditions of CS/HA scaffold. For this aim, the CS, CS/HA scaffolds, and CS/HA scaffolds combined with mesoporous SiO_2-HA particles were fabricated through the freeze-drying method. They assessed their bone regeneration capacity for curing critical-sized bone defects in rats. The findings may be promising for introducing new kinds of scaffolds for bone regeneration in tissue engineering in future therapeutics to be used as implant devices on bone injury sites.

Section snippets

Materials

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), absolute ethyl alcohol, $Ca(NO_3)_2.4H_2O$, and $NH_4H_2PO_4$ were purchased from Merck, Germany. Chitosan (medium molecular weight (200 kDa), 75–85 % deacetylated), acetic acid, ammonium hydroxide solution, RPMI-1640 medium (R5886), fetal bovine serum (FBS), penicillin, phosphate-buffered saline tablet (PBS), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), trypsin (0.25 % EDTA solution), paraformaldehyde,

Characteristics of HA and mesoporous SiO_2-HA particles

Fig. 3a and b demonstrate the FESEM images of HA and mesoporous SiO_2-HA particles, respectively. Based on these images, the distribution of HA particles is broad with a size of about 20–1000 nm. The morphology of mesoporous SiO_2-HA particles is spherical with a mean distribution size of about 200 nm. The presented TEM image of mesoporous SiO_2-HA particles in our previous work [40] proved their porous structure. Figs. 3c and d represent the XRD patterns of HA and mesoporous SiO_2-HA particles,

Conclusion

In the present study, mesoporous SiO_2-HA particles were added to the chitosan (CS)/hydroxyapatite (HA) scaffolds to expedite osteoconductivity, and the scaffolds were

fabricated by the freeze-drying method. In the presence of mesoporous SiO_2-HA particles the CS/HA scaffold was highly porous and interconnected with a pore size of around 145 ± 1.4 µm. Also, the incorporation of mesoporous SiO_2-HA particles could improve the compressive strength of the scaffold and modify the degradation rate.

CRediT authorship contribution statement

Nesa Abdian: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Hamid Soltani Zangbar:** Writing – review & editing, Project administration, Conceptualization. **Mohamadreza Etminanfar:** Writing – review & editing, Project administration, Conceptualization. **Hamed Hamishehkar:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they do not have any competing financial interests or relationships that may have influenced their work.

Acknowledgment

We appreciate the cooperation of the Clinical Research Development Unit of Imam Reza General Hospital, Tabriz, Iran in conducting this research. Tabriz University of Medical Sciences is greatly appreciated for the generous for financial support through grant No. 70976.

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