RESEARCH ARTICLE

A novel investigation on characterization of bioactive glass cement and chitosan-gelatin membrane for jawbone tissue engineering

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ABSTRACT

The aim of this work was to investigate the characterization of a bioactive glass (BG) ceramic prepared by sol–gel technique and chitosan-gelatin membranes prepared by lyophilization technique containing 0, 1 and 2 vol.% ethanol. Early-absorbing and late-absorbing bone substitutes in practice constitute the main volume of bone substitutes used by dentist and orthopedic surgeons. When the graft is to be gradually replaced by normal bone, the important issue is the duration and rate of graft absorption. The prepared samples were characterized using fourier transformed infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis. The presence of Ag nanoparticles into bioactive glass was evaluated. Porous membranes were examined under scanning electron microscopy (SEM) to estimate the size of the pores and analysis the morphological behavior. The pore diameter could be controlled within the range 10–30 µm by adjusting the percent of ethanol. The formation of needle-like hydroxyapatite (HA) crystals on the surface of the membrane after 7 days immersion in the ringer's solution was also assessed using SEM images. The SEM image results illustrated the porous structures in the membranes. The average pore size for chitosan-gelatin membrane with 2% ethanol were 30±6.3 µm. Finally, these obtained results suggest that the developed membrane with 2% ethanol possess the prerequisites for tissue engineering and can be used for jawbone tissue engineering applications.

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INTRODUCTION

Tissue engineering is an emerging technology developed for the regeneration of bone tissues and organs with an increasing interest within the biomaterials field [1-2]. Bioactive glass (BG) ceramic was developed by Hench et al. in 1960s, as a biomaterial to repair for bone defects. The BG is widely used in dentistry and orthopedic applications [3]. BGs ceramics are a group of osteoconductive silicate-based materials used for bone repair that can bond to soft and hard tissue [4]. The bonding ability of these materials is attributed to the formation of carbonated apatite layer on the surface of the materials [5-21]. Calcium phosphates (CaPs) ceramics (i.e. hydroxyapatite (HA), tricalcium phosphate (TCP)) and BGs (silica glasses containing calcium and phosphorus) have proven good biological properties and clinical successes in some specific applications [22-28]. In many tissue engineering strategies, a temporary polymeric-based porous scaffold is employed as a substrate for initial cell attachment and as a physical support to guide new tissue formation [29-34]. A major step in this procedure is the rapid formation of fibrous tissue around the implanted scaffold, which leads to the development of a necrotic core due to the limitations for cell penetration and nutrient exchange [35-41]. Therefore, a key factor in tissue engineering is the design and control of the surface

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properties, in order to enhance the bonding of the implant to the tissue [42-50].

It has been recognized that some ceramics, used as artificial implants for bone defects, could spontaneously bond to living bone without the formation of surrounding fibrous tissue like BG [10], sintered HA [11, 51-57] and sintered β-tricalcium phosphate (β-TCP) [12]. The sol–gel technique, as a chemical method provides an available technique to synthesize BGs. Compared with the traditional melting method, the sol–gel technique has the advantage of lower processing temperature and allows us to obtain glasses with higher purity and chemical homogeneity and composition control [13]. Moreover, sol–gel processed material has better bioactivity due to higher surface area and porosity features. Sol–gel processing, involves the synthesis of a solution (sol), typically composed of metal organic and metal salt precursors followed by the formation of a gel by chemical reaction or aggregation, and lastly thermal treatment for drying, organic removal, and sometimes crystallization process [14].

There has been an increasing interest in the use of natural based macromolecules in tissue engineering [15–17]. Natural polymers including collagen, alginate, agarose, fibrin, and chitosan is widely used in tissue engineering application with sufficient mechanical performance either experimentally or analytically using various simulation software [18-25, 58-60]. These materials are known to be biocompatible, and they exhibit an environment that resembles the highly hydrated state of natural tissues [18]. In the recent years, considerable attention has been given to chitosan-based materials and their applications in the field of tissue engineering. It can be obtained by partially deacetylating of chitin which can be extracted from crustacean. It is a polysaccharide composed of glucosamine and N-acetyl glucosamine linked with a β1-4 glucosidic linkage. Chitosan is biocompatible and can be degraded by enzymes in human body, and the degradation product is nontoxic [19]. The chitosan has been studied in many biomedical fields, including bone tissue engineering [20–22], blood vessel [23] and nerve system [24].

However, the main issues with natural polymers are their weak mechanical properties, lack of cellular interactions, and uncontrolled degradation [32-47]. For the increase of mechanical property [47-53], some composites of polymer have been developed for bone tissue engineering and other fields [52-57]. Gelatin polymer is added to the membrane system to enhance the physical and mechanical properties [26, 27]. The bioactivity of chitosan-gelatin membrane needs to be improved for specific tissue like most of polymers. For the improvement of the bioactivity of the membrane, it is often combined with other bioactive materials. As a major inorganic component of natural bone, HA is a biomimetic material with good biocompatibility and bioactivity in bone tissue engineering. It was reported that the addition of HA in the polymer composites could improve the activity and viability of cells cultured on them [28], or improve both the mechanical and cell-attachment properties of the membrane [29]. Chitosan-gelatin membrane can be molded into various forms and can form a porous structure with lyophilization [30]. In this article, we describe the preparation of bioactive glass cement and chitosan-gelatin membrane and investigate their properties relevant to jawbone tissue engineering applications.

MATERIALS AND METHODS

The materials used in sol-gel derived BG cement and chitosan-gelatin membrane are listed in Table 1 and 2, respectively.

Synthesis of bioactive glass cement

Bioactive glass cement with composition SiO_2 (64%), CaO (26%), P_2O_5 (8%) and $AgNO_3$ (2%) was synthesized by the sol–gel method. In the first step, the solution was prepared as follows: tetraethyl orthosilicate was added into nitric acid (0.1 mol). The mixing process was allowed to be continued for 4 hours at room temperature for the hydrolysis.

Table 1. Materials used in preparation of bioactive glass cement and respective chemical formula and manufacturers.

Material	Chemical Formula	Manufacturer
Tetraethyl Orthosilicate	$SiC_8H_{20}O_4$	98%, Merck; No.8006581000
Triethyl Phosphate	$C_6H_{15}O_4P$	99%, Merck; No.8211411000
Calcium Nitrate Tetrahydrate	$Ca(NO_3)_2.4H_2O$	68%, PROLABO No.22384298
Silver Nitrate	$AgNo_3$	98.5%, Merck
Chloridric Acid	HCl	37%, Merck
Nitric Acid	HNO_3	65%, Merck

Table 2. Materials used in preparation of chitosan-gelatin membrane and respective manufacturers.

Material	Manufacturer
Acetic Acid	Merck-K14155956
Ethanol	Merck
Chitosan	Chitotech
Gelatin	Scharlau-GE0020

Table 3. Composition of ringer's solution.

Materials	Weight (gr)
NaCl	0.86
KCl	0.83
$CaCl_2$, $2H_2O$	0.033

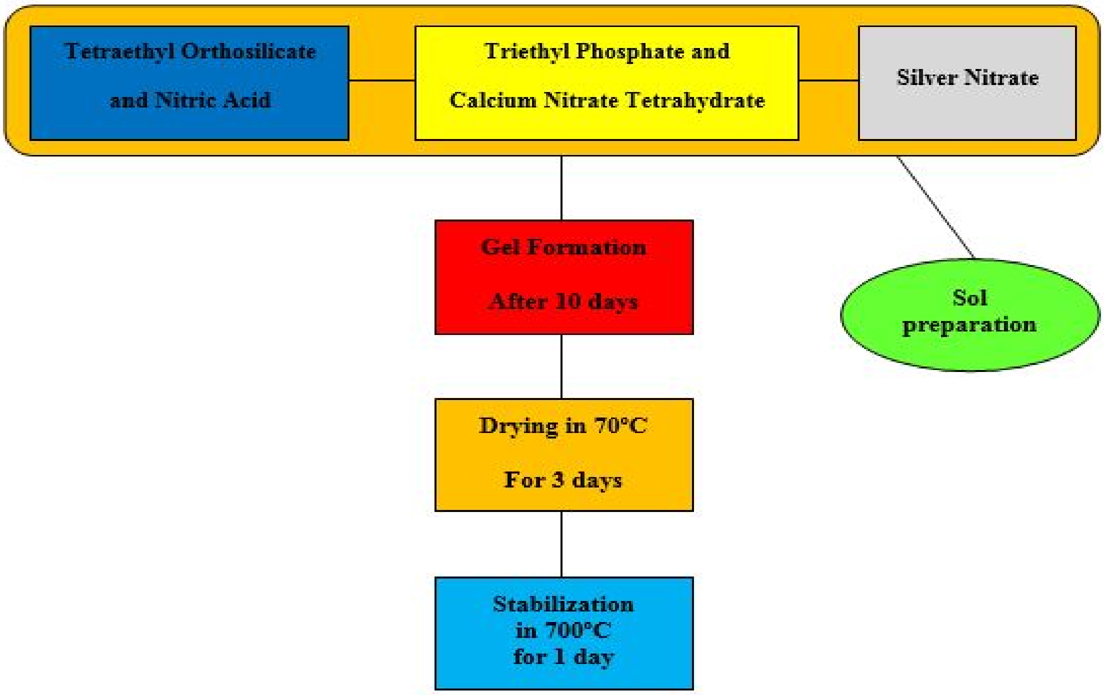

Fig. 1. Flowchart for preparation of the bioactive glass cement.

Triethyl phosphate and calcium nitrate tetrahydrate were added to the solution and stirred for 1 hour. Table 3 shows the ringer's solution composition. After that, silver nitrate was added to solution and stirred for 1 hour. To allow completion of the hydrolysis reaction, mixing was continued for 1h after the last addition. Then the solution was kept at 37°C for 10 days for gelatinize. To get rid of the water, the gel was heated at 70°C for 3 days. The dried gel was then heated to 700°C at 3°C/min and reminded in 700°C for 1 day for stabilization. At last, the powder of bioactive glass was prepared in a planetary ball mil (Retch PMA, Brinkman, USA) for 30 min. The flowchart for the preparation of the bioactive glass cement is illustrated in Fig. 1.

Preparation of chitosan-gelatin membrane

For preparation of chitosan-gelatin membrane, 2 wt.% chitosan-gelatin (with mass ratio of 70/30) was added to 20ml distilled water and stirred to get transparent solution. Two samples were obtained by adding 1 and 2 vol.% ethanol to solution. A sample without ethanol was used as the control. Then the samples were freeze-dried. The flowchart for the preparation of the membrane is illustrated in Fig. 2.

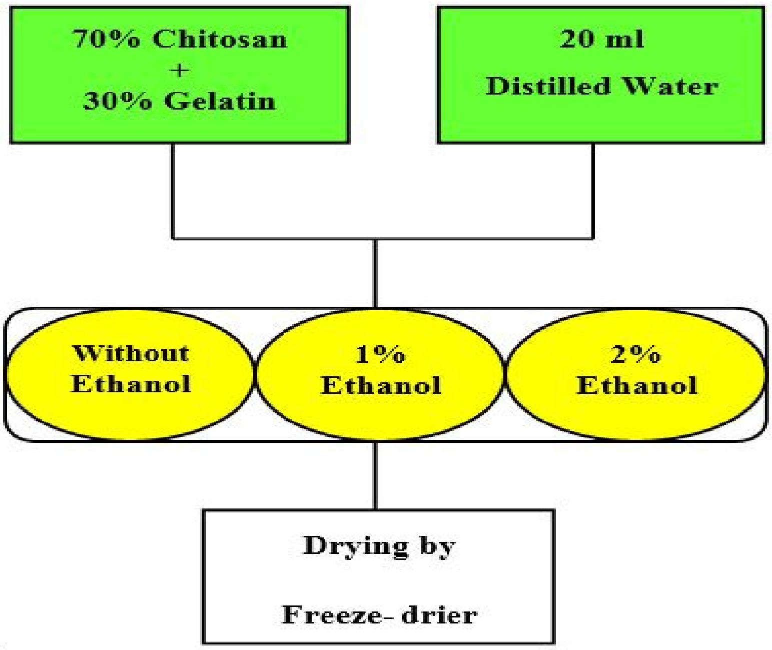

Fig. 2. Flowchart for preparation of the chitosan-gelatin membrane.

Characterization

Fourier-transformed infrared spectroscopy analysis

The powder of bioactive glass cement and three chitosan-gelatin membrane samples (with 0, 1 and 2 vol.% ethanol) were examined by Fourier transform infrared with a Thermo Nicolet FTIR spectrometer. At first 3 mg of the samples were carefully mixed with 800 mg of KBr (infrared grade) and palletized under a vacuum. Then the pellets were analyzed in the range of 400 to 4000 cm^{-1} at a scan speed of 23 scan/min with a 4 cm^{-1} resolution.

X-ray diffraction analysis

The phase composition of the bioactive glass cement was analyzed using X-ray diffraction technique (XRD) with a Philips PW 3710 diffractometer. This instrument was used with voltage and current settings of 30 kV and 25 mA respectively and used Cu-Kα radiation (1.540510Å). For qualitative analysis, XRD diagrams were recorded in the interval $10° \leq 2\theta \leq 70°$ at a scan speed of 2°/minute.

Scanning electron microscopy analysis

The chitosan-gelatin membrane samples were coated with a thin layer of gold (Au) by sputtering (EMITECH K450X, England) and then the shape and morphology of prepared membranes were observed on a scanning electron microscope (SEM; STEREOSCAN S 360 Cambridge) that operated at the acceleration voltage of 20 kV.

RESULT AND DISCUSSION

FT-IR analysis

Fig. 3 (a-d) shows the FT-IR spectra of BG powder. The characteristic bands (listed in Table 4) exhibited in the sample spectra assigned here: (a) three bands were observed at 450 cm^{-1}, 820 cm^{-1} and 870 cm^{-1} due to the vibrational mode of δ(Si–O–Si), stretching mode of Si-O-Si and Si-OH respectively. (b) The band at 800 cm^{-1} arises from SiO_4 and the band at 570 cm^{-1} arises from Si-O. (c) The bands at 700 cm^{-1}, 850 cm^{-1} and 890 cm^{-1} arise from silica particles in the structure. (d) The band at 1480 cm^{-1} refers to change in H-O-H groups due to an interaction of its hydrogen bond with silanol groups. Therefore, according to these explanations, it is obvious that the synthesized powder is certainly BG cement.

XRD analysis

Characterization of the bioactive glass was done with XRD. The XRD analysis was performed using the X-ray diffractometer. Fig. 4 shows the XRD pattern of the bioactive glass. The straight base line and the sharp peaks of the diffractogram in

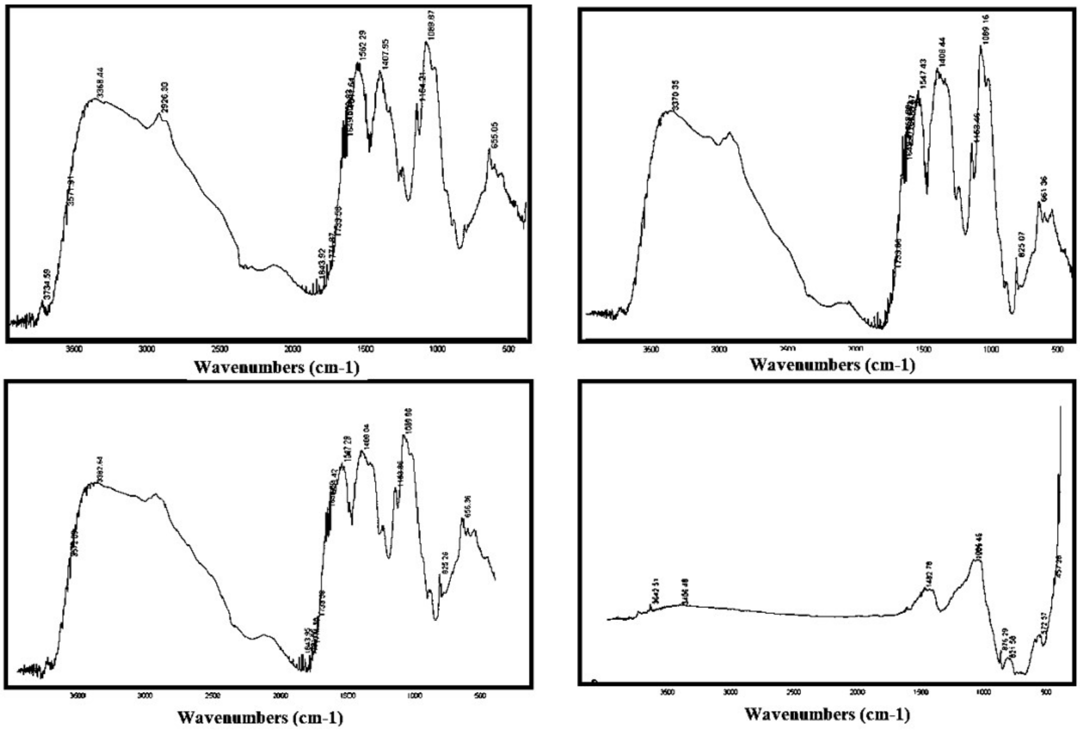

Fig. 3. FTIR spectra of the bioactive glass powder.

Table 4. Infrared assigned for the synthesized bioactive glass powder.

Infrared frequency (cm^{-1})	Assignment
3440	$(H_2O)\upsilon$
1635	$\delta(H_2O)$
1390, 1450	$\upsilon(No^{-۳})$
1070, 1200	υ_{as}(Si–O–Si)
950	υ(Si–OH)
798	υ_s(Si–O–Si)
570	υ(Si–OH)
450	δ(Si–O–Si)

the figure confirmed that the product was well crystallized. It can also be seen in this figure, that the peaks corresponding to silver (i.e. 38, 44.3, 64.5, and 77.5) are clearly identified.

SEM observations

Scanning electron microscopy was used to examine the chitosan-gelatin membranes involving 0, 1 and 2 vol.% ethanol and to observe the morphology of them, especially needle-like HA nanocrystals in the emerged specimen in ringer's solution and to estimate the shape and diameter of porosities. SEM micrographs of the membranes are shown in Fig. 5. As the percent of ethanol increased, SEM results showed an increase in porous size. Meanwhile, as can be seen, the porosity shapes become spherical forms with enhancing the percentage of ethanol. The SEM images revealed the formation of nano-sized needle-like HA crystals on the surface of the membrane after 7 days immersion in the ringer's solution as shown in Fig. 5(a-d).

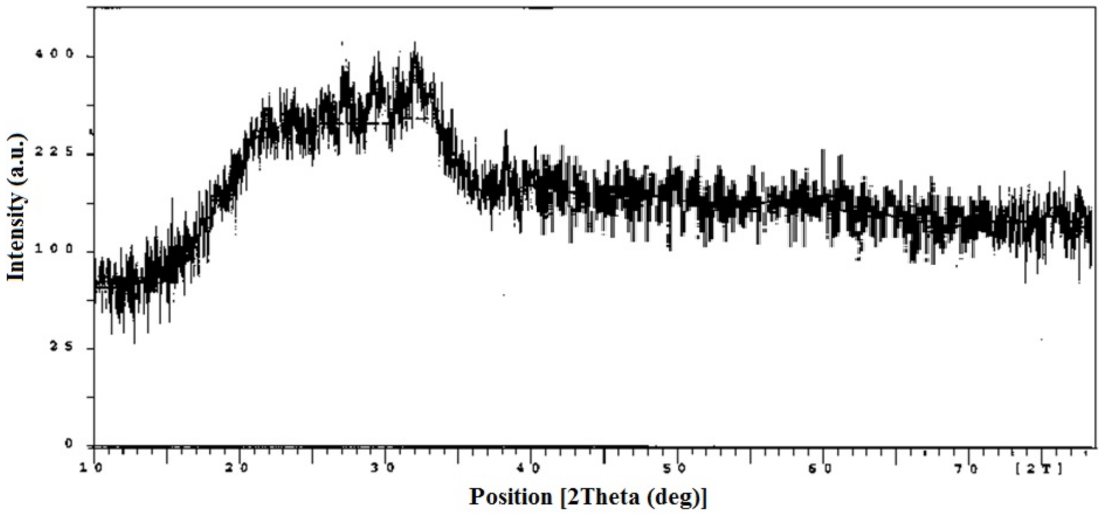

Fig. 4. XRD pattern of the BG cement.

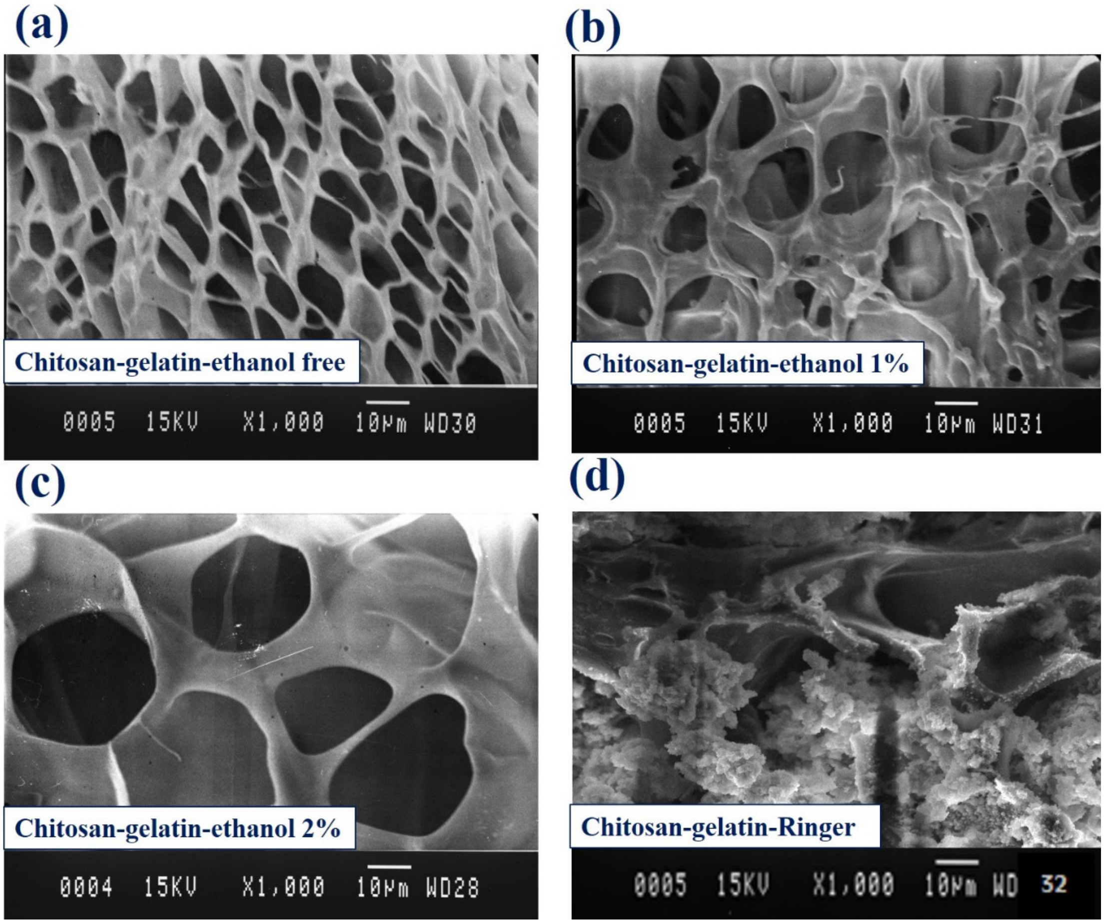

Fig. 5. SEM image of (a) the chitosan-gelatin membrane without ethanol, (b) with 1% ethanol, (c) with 2% ethanol and (d) following 7 days incubation in ringer's solution

CONCLUSIONS

In conclusion, we prepared bioactive glass cement consisting of silver and chitosan-gelatin membrane for reconstruction of jawbone. The FTIR analysis showed all the typical absorption characteristics of BG. Ag particles into the bioactive glass composition were verified by XRD patterns representing peaks characteristic of Ag nanoparticle. Moreover, the cement was well crystallized. The SEM images results illustrated the porous structures in the membranes. The average pore size for chitosan-gelatin membrane with 2% ethanol were 30±6.3 µm. The membrane with optimum percentage of ethanol is very suitable for tissue engineering with sufficient pore size for cells to infiltrate. One of the collagen disadvantages as a membrane is that collagen decomposed before the process of bone formation completely and the process of its decomposition and absorption accompanied by a slight inflammation in the formed tissue. Collagen uptake is an enormous process in which collagenase and gelatin is involved in different stages of collagen membrane breakdown. Histological investigation has shown that slight inflammation in the formed tissue does not interfere with the degeneration and absorption of collagen in the osteogenic process.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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