Collagen-Apatite Nanocomposite Membranes for Guided Bone Regeneration

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Abstract: Collagen-apatite nanocomposite is regarded as a potential biomaterial because of its composition and structure, which are similar to those of human hard tissues. However, there have been few investigations of its mechanical and biological benefits in direct comparison with a collagen equivalent. Herein, we successfully produced a biomedical membrane made of a nanocomposite, and systematically evaluated the mechanical, chemical, and biological properties of the nanocomposite in comparison with those of pure collagen. The results showed that significant improvements were achieved by the nanocomposite approach, particularly in terms of the mechanical strength and chemical stability. The present findings point to the potential usefulness of the collagen-apatite nanocomposite membrane in the field of guided bone regeneration (GBR). © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 83B: 248–257, 2007

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INTRODUCTION

Significant progress has been made in the field of biomaterials and tissue engineering, driven by the increasing demands on the reconstruction of defective tissues. Many advanced clinical treatments have been developed, using novel biomedical materials, enabling the successful repair and regeneration of human tissues. Guided bone regeneration (GBR) is one of the most promising strategies to repair and regenerate specific type of tissues, particularly bony tissue. Biomedical membranes placed in a periodontal pocket under the oral mucosa have been found to act as an efficient barrier against aggressive epithelial cell attachment and connective tissue formation, guiding the successful regeneration of calcified bony tissue. Many studies have been conducted to identify better functional materials, which can act as a membrane to guide and regenerate bony tissues. However, most of the candidate materials developed thus far did not satisfy all of the properties required for GBR membranes, which include mechanical strength, biological affinity, and chemical stability.

For example, poly(tetrafluoroethylene) (PTFE) and Ti mesh, which have been tested over the past decade, are basically inert, and require secondary surgery to remove the membrane. Moreover, these membranes have a very limited critical defect size for which they can heal and generate new tissues. To overcome these problems, resorbable membranes made of materials such as collagen and synthetic biodegradable polymers have been proposed and studied by several researchers. Collagen membranes have shown good bio-affinity and resorbability. However, major concerns have been raised with regard to their poor mechanical strength and too fast dissolution rate. Many recent studies of collagen membranes have reported unsatisfactory in vivo results, for example, the regeneration rate of bone was not as effective as that obtained with nonresorbable PEFE membranes, mainly because of the difficulty in producing collagen membranes with high mechanical and biological properties. The main alternative membrane materials are degradable synthetic polymers, such as poly(lactide) and poly(lactide-co-glycolide). However, they are not the optimal choice for GBR membranes either, because of their poor cell affinity and compatibility, although they have sufficient mechanical properties as compared with collagen.¹⁻⁵

Recent studies have suggested using composites of osteoconductive calcium phosphate ceramics and biodegradable synthetic polymers. For example, hydroxyapatite/poly(ε-caprolactone) composites,⁶ biodegradable polyactive⁷/apatite membranes,⁷ and β-Tricalcium phosphate and poly(l-lactide-co-glycolide-co-ε-caprolactone) composites⁸ have been proposed. Although their feasibility for GBR was often reported to be improved by the composite approach, one major drawback in these systems is the presence of large-
sized ceramic particles and their nonuniform distribution within the polymer matrix, leading to mechanical degradation and the creation of particle debris. Nevertheless, the involvement of bioactive ceramics in the degradable polymeric phase has been considered to stimulate the osteoblastic responses and improve the bone forming ability.

In this respect, one promising approach to generate bone regenerative membranes is to organize the apatite nanoparticles in concert with a polymeric matrix. The apatite/collagen system is considered to be promising when organized well on the nanoscale, because apatite and collagen are the major inorganic and organic constituents of human hard tissues, respectively. Consequently, this system is very close to the composition and structure of bone. Many studies have been carried out on the apatite/collagen system, however most of them were primarily concerned with the control of reaction variables and evaluation of chemical and structural properties.

Moreover, most works produced collagen-apatite in the form of dried powder, or in its pressed bulk form, to optimize its mechanical properties. A novel synthesis process was developed by Wang et al. to prepare HA/collagen composite, in which nanometer sized HA is homogeneously dispersed in collagen matrix. The presence of insoluble collagen was found to have a function of regulating the distribution of HA particles.

However, very little research has been carried out on the type of membrane, which is specifically useful for the GBR applications. This is mainly due to the difficulty involved in converting the collagen-apatite into the type of membrane, whose mechanical and biological properties are satisfactory for practical applications. In conventional approaches, wherein a thin membrane with a thickness of the order of several hundreds of micrometers is used, the collagen/apatite composite did not exhibit sufficient mechanical strength. As a result, no reliable data is available on the mechanical properties of collagen/apatite membranes and, furthermore, there have been no reports on their biological stability and clinical potential as a GBR in direct comparison with the pure collagen equivalent. In a recent work by Liao et al., a layered structure composed of apatite-collagen/collagen/PLGA was proposed as a guided regeneration membrane. The membrane showed relatively high tensile strength, however, this was mainly by the effect of PLGA composition. Although the membrane was elegantly engineered, no systematic study on the effect of composition, particularly of the apatite inorganic phase, was carried out.

In the present study, we successfully formulated a collagen/apatite nanocomposite into a thin membrane form (~hundreds of micrometers), using a newly-developed coprecipitation and dynamic filtration technique. Moreover, we investigated the mechanical and biological properties of the nanocomposite membrane, to provide data which were comparable with those of the pure collagen equivalent. Finally, we discuss the potential utility of this collagen/apatite membrane in GBR applications.

**MATERIALS AND METHODS**

**Coprecipitation of Collagen/Apatite**

The procedure used for the coprecipitation of the collagen/apatite nanocomposites and their formulation into a membrane is schematically illustrated in Figure 1(a). Firstly, the coprecipitation process of collagen and apatite was slightly modified from our previous work. As the starting materials, Ca(OH)₂ (99.995%, Aldrich, USA), H₃PO₄ (99.999%, 85 wt% aqueous solution, Aldrich), and Type I collagen (ateolocollagen from bovine skin with pepsin treatment, MW 300,000, RegenMed, Korea) were used. Ca(OH)₂ was completely dissolved in cold distilled water, after taking into consideration the solubility limit, which was calculated from their thermodynamic parameters. Separately, collagen was dissolved in H₃PO₄ solution (59.7 mM diluted in distilled water). Both the Ca(OH)₂ and H₃PO₄/collagen solutions were added to a reaction vessel containing Tris/HCl buffer solution adjusted to pH 9 at 37°C. During the reaction, care was taken to maintain the pH at 9 using HCl and NH₄OH. The amounts of Ca, P, and collagen were determined to produce final ratios of collagen/apatite of 80/20.
and 60/40 (wt/wt). The mixture was stirred vigorously for 48 h at 37℃ in a water bath. Part of the coprecipitated products was gathered for further evaluation.

Fabrication of Nanocomposite Membrane

The coprecipitated product was formulated into a thin membrane form by means of the dynamic filtration technique, as illustrated in Figure 1(b). When the reaction was complete, the precipitates were poured into a filtration system under vacuum, wherein the cellulose filter was kept within a polystyrene vessel with continuous agitation to aid the filtration process and to produce a membrane with homogeneous composition and uniform thickness. The filtration process was followed by repeated washings with distilled water. When the coprecipitates had settled down to a uniform thickness, the filter cake was freeze-dried. A pure collagen membrane was made by the same dynamic filtering method to act as a reference.

The freeze-dried membranes were chemically cross-linked, using 1-ethyl-3-(3-dimethyl aminopropyl)carbodi-imide (EDC) and N-hydroxysuccinimide (NHS). To accomplish this, the membranes were immersed in 100-mM EDC/100-mM-NHS solution for 24 h at room temperature. The cross-linked membranes were washed sufficiently with distilled water five times for 5 min each to remove the residual EDC and NHS. After washing, the membranes were refreezed and lyophilized at −60℃ under a vacuum.

Following their fabrication, the membranes were warmpressed at 40℃ in a moist atmosphere and then air-dried for 2 h under controlled pressure. The membranes were sterilized with 70% ethanol for 3 h and then lyophilized at −60℃ under a vacuum. In particular, the membranes were prepared with a range of thicknesses (∼200–1000 μm) by adjusting the filtration process. Figure 1(c) presents an image of the produced collagen/apatite membranes after being cut to appropriate dimensions.

Characterization

Thermogravimetric analysis (TGA) was performed to determine the amount of apatite in the nanocomposites. The membranes, each with a weight of 20 mg, were studied using a TG analyzer (TGA-1000, Rheometric Scientific, UK) and the measurements were recorded from 30 to 900℃ with a heating rate of 10℃/min in air. The remnants were inorganic components, later identified as hydroxyapatite by XRD analysis. The chemical analysis of the nanocomposites was conducted, using a Fourier transform infrared (FTIR) spectrometer ( Nicolet Magma 550 series II, Midac, USA) over the wavelength range of 4000–400 cm⁻¹ at a resolution of 1 cm⁻¹ with an average of 64 scans. The microstructure of the nanocomposites was observed by field-emission scanning electron microscopy (FE-SEM, JSM-6330F, JEOL, Tokyo, Japan). The internal structure of the nanocomposites was examined by transmission electron microscopy (TEM, CM-20, Philips Electron Optics, Netherlands). The density of the membranes prepared with different thicknesses was measured. As the membranes had initially the same weight, their density was predominantly dependent on the thickness, that is, the thicker the specimen the lower the density. The exact thickness of the specimens was measured, using a confocal laser scanning microscope (CLSM, OLS1200, Olympus). The density of the samples was then calculated from their dimensions and weight.

Mechanical and Swelling Tests

Mechanical tests were carried out using a universal testing machine (Model 5565, Instron, Danvers, MA). The membranes with thicknesses in the range of ∼550–660 μm were cut into a dumbbell shape with a gauge length of 25 mm and an inner width of 5 mm. A tensile force was applied at an extension rate of 1 mm/min. At least six specimens were tested for each condition.

The degree of swelling of the membranes (thickness of ∼600 μm) was studied by a volume change after soaking in distilled water at room temperature for 24 h. After their removal, the membranes were laid on filter paper for 1 min until no more dripping water was observed. The volume of the swollen membranes was measured and the percentage volume change was calculated, using the following equation: volume change (%) = [(V s − V d)/V d] × 100, where V d and V s are the volumes of the dried and swollen membranes, respectively.

Degradation Test in Collagenase

The biological stability of the membranes was evaluated by means of in vitro collagenase biodegradation test. The membranes with a thickness of ∼600 μm and a weight of ∼2 mg were incubated for 1 h in 1 mL of phosphate buffered saline (PBS, pH 7.4). Subsequently, 300 U of type I collagenase (300 U/mg, Sigma) in 1 mL of PBS (pH 7.4) was added. After incubation for 24 h at 37℃, the degradation was stopped by immediately precipitating the assay in an ice bath. Following centrifugation at 1500 rpm for 10 min, the clear supernatant was hydrolyzed with 6M HCl at 110℃ for 24 h. The amount of hydroxyproline released from the collagen molecules in the membranes was measured, using the ELISA method. Aliquots of supernatant (50 μL) were added to 100 μL of 1.4% chloramine T (Aldrich) in acetic-acid buffer, with pH 6.0. Subsequently, 100 μL of Erlich’s solution [1M p-dimethylaminobenzaldehyde (Aldrich) in 20% perchloric acid] was added and allowed to incubate at 65℃ for 15 min. Absorbance was measured at 540 nm, and the amount of hydroxyproline was determined against a standard curve generated, using known concentrations of hydroxyproline (Aldrich).

The degree of biodegradation is defined as the percentage of hydroxyproline released from the membrane, as compared with that released from a completely degraded one with the same composition and weight. Also, the amount of degraded...
collagen in the solution was measured, to determine the effect of apatite on the degradation process.

**Preliminary Cellular Assay**

The murine-derived pre-osteoblast MC3T3-E1 cell line (ATCC, CRL-2593) was cultured in regular culture media consisting of α-modified minimum essential medium (α-MEM; Join Bio Innovation, Seoul, Korea) supplemented with 10% heated-inactivated fetal bovine serum (GIBCO, USA) and 1% antibiotic/antimycotic (GIBCO) in a humidified atmosphere containing 5% CO₂ at 37°C. Before the experiments, the cells were trypsinized and plated on the specimens at a density of 1.5 × 10⁴ cells/cm² and cultured in an osteogenic medium [the regular culture media described above plus 10 mM β-glycerol phosphate and 50 μg/mL L-ascorbic acid (Sigma, USA)]. After harvesting them at 3 days, the cell viability was measured as the mitochondrial NADH/NADPH-dependant dehydrogenase activity, using a cell proliferation assay kit (CellTiter 96 Aqueous One Solution, Promega, USA). Then, the culture medium was removed and 100 μL of MTS solution (3-(4,5-dimethylthiazol-2-yl)-5-(3-caebozymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; CellTiter 96 AQueous One Solution kit, Promega) in 1 mL of culture media was added to each well and the wells left to stand for 3 h. Finally, the colorimetric measurement of a 200 μL sample of each solution was performed on a spectrophotometer at 490 nm. The cell morphology at 3 days was observed with SEM after fixing with glutaraldehyde (2.5%) for 10 min, dehydrating with graded ethanol (75, 90, 95, and 100%) for each 7 min, critical point drying for 20 min and gold coating.

**Statistical Analysis**

Data are expressed as mean ± 1 standard deviation (SD). The statistical analysis was performed, using a two-population Student’s t-test. The p values <0.05, <0.01, and <0.001 were considered to be significant depending on the test.

**RESULTS**

**Phase and Structure**

The apatite content in the collagen/apatite membranes was confirmed by the TGA analyses, as shown in Figure 2(a). During heating, weight losses were observed for all of the membranes, apparently because of the removal of the organic components. There was no significant difference in the TG curves depending on the composition, with the first weight loss of 5~10% occurring at 50~100°C due to the evaporation of moisture, and the second weight loss at 240~250°C associated with the decomposition of collagen. The mineral residue contents (weights at 700°C) were found to be slightly different from the amounts of minerals that were initially added, namely 22 and 34% of the remaining minerals for the 20 and 40%-apatite membranes, respectively. These composition values (22 and 34%) are the corrected values, after taking the remnant of pure collagen (~5%) into consideration.

Figure 2(b) shows the FTIR spectra of the membranes. Typical IR bands for collagen were observed; N/C0/C0H stretching at ~3310 cm⁻¹ for the amide A, C/C0/C0H stretching at ~3063 cm⁻¹ for the amide B, C=O stretching at 1600~1700 cm⁻¹ for the amide I, N=H deformation at 1500~1550 cm⁻¹ for the amide II, and N=H deformation at 1200~1300 cm⁻¹ for the amide III bands. It was reported that the spectral features of the amide B band arising from the C=H stretching were considerably influenced by the cross-linking. In the collagen/apatite nanocomposite membranes, typical phosphate bands (PO4³⁻) associated with the apatite structure appeared at around 1097~1110 cm⁻¹ and 1030~1033 cm⁻¹ (for ν3 mode) and around 601~607 cm⁻¹ and 563~569 cm⁻¹ (for ν4 mode).

**Morphology**

The morphologies of the membranes are shown in Figure 3. The surface of the pure collagen membrane was smooth and almost fully dense [Figure 3(a)]. In the nanocomposite
membrane (20% apatite), the surface was observed to be rough and contain some submicron-sized pores [Figure 3(b)]. However, the cross-section of the membranes (both collagen and nanocomposites) had a very porous structure, wherein the pores, whose sizes ranged from tens to hundreds of micrometers, were aligned along the lateral direc-

Figure 3. Electron micrographs of the membranes: (a) SEM surface image of pure collagen, (b) surface of collagen with 40% apatite, (c) cross-section of collagen with 40% apatite, (d) magnification of (c), and (e) TEM image and SAD pattern of collagen with 40% apatite.
tion [Figure 3(c)]. This pore configuration was similarly observed in all of the membranes. A closer examination showed the presence of a fibrillar structure constituted of collagen fibers and precipitated apatite nanoparticles on the collagen surface [Figure 3(d)]. The TEM image of the nanocomposite [Figure 3(e)] revealed the internal structure, wherein numbers of apatite nanocrystallines with an elongated shape were well precipitated within the collagen matrix. The selected area diffraction pattern of the crystalline showed typical diffused rings with highlighted dots corresponding to specific lattice planes [(002) and (211)].

**Density**

Membranes with different densities were prepared by controlling their thickness. For three different weights of pure collagen, viz. 2.0, 3.07, and 4.39 mg, membranes were prepared with 0, 20, and 40% apatite, respectively, and each type of membrane was produced with a range of thicknesses (≈200–1000 μm) by adjusting the filtration process. As a result, membranes were prepared with the same composition and initial weight but with different densities and porosities. The density of the membranes was calculated by measuring their weights and dimensions [with an area (A) of 25 mm and variable thicknesses (t)], as shown in Figure 4 (presented as symbols). The densities were observed to decrease as the thickness increased, which indicates that the porosity increased with increasing thickness.

**Mechanical Properties**

In Figure 5, the typical stress–strain curves for each type of membrane are presented. Specimens with average thicknesses of ≈550–650 μm were tested. All of the membranes exhibited a similar stress–strain pattern, that is, the stress increased linearly (elastically) with respect to the strain at first and then deviated from linearity as the strain increased. As the amount of apatite increased, the membrane exhibited a higher stress at failure and initial slope, but a lower strain at failure.

The mechanical properties of the membranes, such as their tensile strength, elastic modulus, and strain at failure, are summarized in Figure 6(a–c). To minimize the variation in the mechanical properties of the membranes at a given composition, which are dependent on their density (porosity), samples with thicknesses in the range of ≈500–600 μm were tested.

The tensile strengths of the nanocomposite membranes were approximately four (1.55 MPa for the collagen membrane with 20% apatite) and six (2.37 MPa for the membrane with 40% apatite) times that of the pure collagen membrane (0.43 MPa). Moreover, the elastic moduli of the nanocomposite membranes (42 and 83 MPa for the membranes with 20 and 40% apatite, respectively) were also significantly increased as compared with that of the pure collagen membrane (9 MPa). However, the strain at failure was lower in the nanocomposite membranes (5.7 and 4.1% for the specimens with 20 and 40% apatite, respectively) than that in the pure collagen membrane (7.8%). The nanocomposite membranes showed significant improvements in their strength and elastic modulus, but a moderate reduction in their strain at failure.

**Degree of Swelling**

The degree of swelling was examined by measuring the volume difference before and after soaking the membranes in distilled water for 24 h. While the pure collagen membranes swelled to more than twice the volume (mainly thickness) of their initial state, the nanocomposite membranes were observed to retain their initial volume (thickness) well, as shown by the swollen morphologies of each membrane in Figure 7(a). The thickness changes of the swollen membranes were measured and the results are shown in Figure 7(b), which demonstrates that the nano-

![Figure 4](image-url)  
**Figure 4.** Density of the membranes measured within a range of thicknesses (≈200–900 μm). The density is proportional to the inverse of the thickness of the membrane at a given composition.

![Figure 5](image-url)  
**Figure 5.** Typical stress–strain curves of the membranes under tensile load.
composite membranes retained their initial thickness better (significantly different at $p < 0.05$).

### Enzymatic Stability

The chemical stability of the membranes was measured by assessing their resistance to collagenase medium. Figure 8(a) shows the images of the membranes in the vials during the biodegradation test conducted for 24 h. The pure collagen membrane was completely degraded, while the membrane with 20% apatite nanocomposite collapsed to the point that its initial shape was no longer recognizable. However, the membrane with 40% apatite appeared to retain its integrity well against the enzymatic solution. The degree of biodegradation of the membranes was quantified by a hydroxyproline assay after normalizing the results to a standard composition and weight, as shown in Figure 8(b). The degree of collagen degradation was expressed in terms of both the amount of collagen remaining in the membrane and the total weight of the membrane. The degree of collagen degradation was reduced by about 20% for the nanocomposite membrane containing 20% apatite and about 50% for the membrane containing 40% apatite. This improvement in the biodegradation behavior due to the presence of apatite was more significant when considering the degradation of the whole membrane.

### Cell Viability

The nanocomposite membranes developed in this study maintained favorable bone-derived cellular responses, as preliminarily assessed by their cell growth morphology and proliferation rate. Figure 9(a) shows the electron micrograph of the cells grown on the nanocomposite membrane with 20% apatite after 3 days of culturing. The cells were observed to grow favorably on the membranes, with active...
cytoskeletal extension and strong adhesion onto the substrate. A similar cell growth morphology was observed on the other membranes. The cell viability on the membranes was measured by an MTS method after culturing for 3 days, as shown in Figure 9(b). The cell growth level on the nanocomposite membranes was similar to that on the pure collagen membrane, without any significant difference ($p > 0.05$).

**DISCUSSION**

We reported herein the potential usefulness of the collagen-apatite nanocomposite for GBR applications. Although several polymeric systems have been proposed for use as a GBR membrane, such as PTFE, PLA, and collagen, they do not satisfy all the properties required for the successful regeneration of bony tissue. For example, PTFE is not resorbable, although it has sufficient mechanical strength. PLA is a degradable polymer, but it exhibits poor cellular attachment. Collagen, which is one of the strongest candidates as a membrane, shows poor mechanical properties, and too high a biodegradation rate.

Compared with these membranes, the collagen-apatite nanocomposite showed mechanical and biological properties, which are closer to those required for the regeneration of hard tissues and, thus, for use as a GBR membrane. In this study, the concentrations of apatite (20 and 40%) hybridized within the collagen fibrils were lower than the normal compositions in the apatite-collagen systems (60–80%) reported thus far. This is necessary for GBR applications, because when the amount of apatite is more than ~50%, the membrane becomes too stiff to handle and operate for surgical implantation.

The membrane form of the coprecipitated nanocomposite was engineered, using a dynamic filtration process [shown in Figure 1(b)], wherein the nanocomposite solution was successfully filtered into a thin membrane with a homogeneous structure and uniform thickness. The collagen-apatite nanocomposite was observed to retain a composi-
tion and structure, which are close to those of human hard tissues. Moreover, the nanocomposite membranes were designed to retain a porous structure. Studies have shown that permeable and porous membranes exhibit better biocompatibility and bone regeneration than their dense equivalents.\textsuperscript{22} In particular, the pores of the membranes in the present study showed a lamella structure aligned in the lateral direction, which was created by a series of stacking processes of the collagen fibril-apatite nanoparticle assemblies and the subsequent freeze-drying step (Figure 3). In spite of their porous structure, the membranes retained fairly good mechanical strength because of this filtration process. In practice when the collagen-apatite solution was directly freeze-dried without the filtration process, the membranes did not show sufficient mechanical stability. On the other hand, when the collagen-apatite solution was fully dried to form membranes under ambient conditions, the membranes were not porous at all.

In this manner, the dynamic filtration process is considered as an effective method of generating porous but strong membranes. In particular, the porosity of the membranes was controllable by adjusting their thickness. The composition of the membranes was tailored by simply changing the initial composition. As such, by the dynamic filtration technique, functional membranes having layered structures with various compositions were able to be generated. Recent research on collagen membranes with a layered structure (dense/porous structure) demonstrated that they have good bone regeneration capacity, wherein the dense layer impeded the growth of the fibrous cells and the porous side induced osteoblastic differentiation.\textsuperscript{5}

In addition to having a porous structure, a GBR membrane should meet certain mechanical and biological qualifications. The rigidity of a membrane determines its space-maintaining capacity for guiding the regeneration of hard tissue.\textsuperscript{1} More importantly, the membrane should have a suitable degradation rate and not show abrupt degradation, to preserve its mechanical stability during the regeneration process.\textsuperscript{4,5} Although collagen membranes were proposed as a good candidate for GBR membranes, they have poor space-maintaining ability, because of their low rigidity and the fact that they biodegrade at a rate more rapid than required.\textsuperscript{23}

The collagen-apatite nanocomposite membranes developed in the present study exhibited more favorable mechanical properties and biodegradation stability. The tensile strength and stiffness of the nanocomposites were significantly improved as compared with those of pure collagen (Figure 6). The apatite nanocrystallines within the collagen structure organized at the molecular level are considered to play a beneficial role in the strengthening of the collagen, which is the same phenomenon as that normally observed in natural mineralized systems, including bones and teeth. A recent study of gelatin-apatite nanocomposite also supports this mechanical strengthening role played by the apatite component when the inorganic phase was distributed and organized well within the organic matrix.\textsuperscript{24}

One particularly important observation regarding the nanocomposite was the significant improvement in its stability against biodegradation. The enzymatic degradation of collagen membranes is affected by the degree of cross-linking.\textsuperscript{25} In this manner, the biodegradation behavior of collagen commonly shows large scatterings depending on the level of cross-linking. In collagen-apatite nanocomposites, the inorganic nanoparticles also affect the degradation behavior of the system.

The degradation of the nanocomposite in a collagenase medium is deemed to be closely related to the accessibility of the enzyme to the collagen fibrils. The enzyme is known to interact with the collagen fibrils and remain bound to the macromolecular aggregate during the degradation process. According to this theory, \( \sim 10\% \) of the collagen molecules in the collagen fibril are accessible for the binding of the enzyme.\textsuperscript{26,27} Hence, the apatite nanocrystallines precipitated on the collagen fibrils may inhibit the binding of the enzyme, because the number of exposed collagen molecules is reduced by the coverage of the apatite component. The greater the number of apatite nanocrystallines in the membrane is, the higher the blocking effect of the enzymatic degradation will be, consequently improving the enzymatic stability (as shown in Figure 8).

Both of the beneficial effects obtained via the nanocomposite approach, namely the improvement in the mechanical properties and biodegradation stability, along with its favorable cell compatibility as preliminarily assessed in Figure 9, support the potential usefulness of the collagen-apatite membrane in GBR applications. Further in-depth biological assessments of the nanocomposite membrane are currently underway, such as \textit{in vitro} tests of bone matrix synthesis and an \textit{in vivo} study of the degradation and bone regeneration rate, to confirm its practical performance.

CONCLUSION

Collagen-apatite (20 and 40\%) nanocomposites were successfully formulated into macroporous membranes by means of coprecipitation and dynamic filtration methods. The nanocomposite membranes exhibited significantly improved mechanical properties (stiffness and tensile strength) and enzymatic stability compared with those of the pure collagen equivalent. These collagen-apatite membranes are considered to be potentially applicable in the GBR field.

REFERENCES


