Guided Bone Regeneration Using Chitosan-Collagen Membranes in Dog Dehiscence-Type Defect Model

Xiaojing Li, MD, [*] *Xinmu Wang, PhD,* [†] *Tengfei Zhao, MD,* [‡] *Bo Gao, MD,* [§] *Yuwen Miao, MD,* [‖] *Dandan Zhang, MD,* [¶] *and Yan Dong, PhD* [#]

Purpose: The purpose of the present study was to compare a newly developed chitosan-collagen membrane (CCM) with a standard collagen membrane (SCM) regarding their effects on guided bone regeneration.

Materials and Methods: The right mandibular premolars and first molar were extracted from 12 beagle dogs. Four months later, acute buccal dehiscence-type defects (4 × 3 mm in height and width) were surgically created after implant site preparation. The defects were randomly assigned to 4 different groups: CCM-1 (weight ratio of chitosan to collagen of 40:1), CCM-2 (weight ratio of chitosan to collagen of 20:1), SCM, and vehicle control. The dogs were sacrificed after 4, 8, and 12 weeks of healing for radiographic examination, histologic observation, and histometric analysis.

Results: The membrane-treated sites showed more bone formation than the control sites, although no statistically significant differences were found between the membrane-treated sites and the control sites for new bone-to-implant contact and new bone-filled area at any point. At 8 weeks, the new bone height for the membrane-treated sites was significantly greater statistically than that of the untreated group ($P < .05$). At 12 weeks, the CCM-1 group showed significantly greater new bone height (1.91 ± 0.25 mm) than the untreated group (1.20 ± 0.34 mm; $P < .05$). However, the CCMs did not show any statistically significant differences compared with the SCMs for any assessed parameter.

Conclusions: The results of the present study have shown that the developed CCMs can enhance bone regeneration and could be a candidate for use in guided bone regeneration.

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Guided bone regeneration (GBR) has been proved to be a reasonably reliable technique for the treatment of insufficient bone volume, such as a dehiscence- or fenestration-type defect around dental implants.[1-6] The basic concept originally described for GBR involved placement of a barrier membrane to create a secluded space around the bone defects to exclude the invasion of fibrous connective tissue and

*MD Student, Department of Prosthetic Dentistry, Second Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China.

†Associate Professor, Department of Oral Surgery, First People's Hospital of Hangzhou, Hangzhou, China.

‡Resident, Department of Orthopedic Surgery, Second Affiliated Hospital (Binjiang Branch), Hangzhou Binjiang Hospital, Zhejiang University School of Medicine, Hangzhou, China.

§MD Student, Department of Prosthetic Dentistry, Second Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China.

‖MD Student, Department of Prosthetic Dentistry, Second Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China.

¶MD Student, Department of Prosthetic Dentistry, Second Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China.

#Associate Professor, Department of Prosthetic Dentistry, Second Affiliated Hospital, Zhejiang University College of Medicine, Hangzhou, China.

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Address correspondence and reprint requests to Dr Dong: Department of Prosthetic Dentistry, Second Affiliated Hospital, Zhejiang University College of Medicine, Jiefang Rd 88, Hangzhou 310009, China; e-mail: yandong_66@163.com

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simultaneously protect the blood clot and promote the in-growth of osteoblasts into the bone defect site during the bone healing period.[7-9] Bone augmentation can be obtained using resorbable or nonresorbable membranes alone or with the aid of various bone substitutes for GBR procedures.[10] Because of the obvious disadvantages of the necessity of a second procedure for membrane removal and the high membrane exposure rate, the replacement of nonresorbable by resorbable membranes would be highly desirable. The most commonly used resorbable membranes have usually been made from collagen derived from porcine or bovine,[11,12] and considerable studies have shown that the application of collagen membranes and bone substitutes combined with the placement of implants will lead to successful rehabilitation of the previously found bone defect around implants.[7,13,14] However, the native collagen will have degraded within a few days, and untreated collagen membranes lack enough stiffness to maintain the space without the use of bone substitutes and tend to collapse.[13,15] Moreover, a recent clinical study showed that once prematurely exposed to the oral environment, the resorbable collagen membranes become rapidly contaminated and degraded by bacterial collagenases, which is not beneficial for soft tissue healing after premature exposure, a process that jeopardizes GBR.[16]

Chitosan, a linear polysaccharide composed of β(1–4)-linked *N*-acetyl-D-glucosamine residues, can be obtained by partial deacetylation of chitin in the solid state under alkaline conditions or by enzymatic hydrolysis in the presence of chitin deacetylase.[17] Chitin is the second most abundant natural biopolymer and is commonly found in the exoskeleton of arthropods, the cuticles of many invertebrates, and in the cell walls of green algae, fungi, and yeasts.[18] Depending on the source and preparation procedure, the molecular weight of chitosan can range from 300 to more than 1,000 kDa, with a degree of deacetylation of 30 to 95%.[19] Notably, chitosan is biocompatible, can be degraded by lysozyme in vivo through hydrolysis of acetylated residues, and the degradation products are nontoxic.[19,20] The degradation of chitosan is inversely related to the degree of deacetylation, and highly deacetylated forms can last several months in vivo.[21] Moreover, chitosan possesses several distinctive biologic properties, including antimicrobial effects[22,23] and hemostatic properties, promoting wound healing[24] and accelerating bone formation.[25] Furthermore, chitosan can be easily processed into membranes, gels, nanofibers, nanoparticles, beads, scaffolds, and sponges.[17] Because of its inexpensive cost, unique biologic properties, and process flexibility, much attention has been paid to chitosan for biomedical applications in tissue engineering. Compared with most of absorbable collagen membranes presently used in clinical practice, chitosan membranes are less expensive and possess better tissue healing effects and bacteriostatic properties.[26] Thus, the combination of chitosan and collagen into a newly resorbable chitosan-collagen membrane (CCM) would be a desirable candidate for the use of GBR, with the capacity to reduce the bacteria contamination, withstand bacterial collagenolytic degradation, support gingival tissue healing, and promote bone regeneration.

Different experimental studies have applied the newly resorbable membrane made from chitosan and collagen for use in bone regeneration therapy.[27-29] One study has shown that chitosan-collagen sponges have excellent cytocompatibility with the mouse osteoblast cell line and promoted growth and differentiation of osteoblasts into the mature stage.[30] Moreover, the chitosan-absorbable collagen sponge (ACS) has been successfully used to treat critical-size bone defects in rat calvarial defects.[28] After 8 weeks of healing, histomorphometric analysis revealed the amount of new bone formation in the chitosan-ACS sponge was significantly greater statistically than that of both the ACS-alone group and the untreated group. These results have resulted in additional research to evaluate the feasibility of CCMs in a more clinically relevant model.

The aim of the present study is to evaluate whether CCMs when used as barrier membranes without any bone substitutes for GBR will result in a greater amount of bone regeneration than standard absorbable collagen membrane (SCM) in a dog dehiscence-type defect model.

Materials and Methods

ANIMALS

Twelve beagle dogs (aged 18 to 24 months, mean weight 14.75 kg) were used in the present study. The Zhejiang University Ethics Committee for Animal Research approved the animal management and experimental protocol. Before the experimental procedures, all dogs were allowed a 2-week adaptation period.

MEMBRANES

The developed test membranes investigated in the present study were CCMs. Preparation of the CCMs was performed using the following procedures. In brief, the compound solutions of 2% chitosan (medium molecular weight, Sigma Aldrich, St Louis, MO) or 1% chitosan acetic acid solution and 0.1% collagen acetic acid solution (C3511, Sigma Aldrich) at a volume ratio of 2:1 were prepared. After sufficient stirring and centrifuging, the suspension of the compound solutions