ORIGINAL ARTICLE

Collagen–Chitosan Barrier Membrane, a Novel, Indigenous, and Economic Material for Management of Periodontal Infrabony Defects – A Case–Control Study

K. Harikumar, K. Nandakumar[1], C. Devadas[2], Susheela Mathew[2]

Department of Periodontics, Government Dental College, Kozhikode, [1]Department of Periodontics, Azeezia Dental College, Kollam, [2]Department of Biochemistry and Nutrition, Central Institute of Fisheries and Technology, Kochi, Kerala, India

ABSTRACT

Objectives: The main objective of periodontal treatment is to resolve the inflammatory lesion in periodontal tissues. However, the contemporary goal has become regeneration of the lost attachment apparatus through guided tissue regeneration (GTR) technique. The GTR membranes currently available in the market are expensive, which in turn warrants the identification, development, and utilization of more cost-effective and biocompatible materials as barrier membranes for GTR. **Materials and Methods:** The present case–control study was conducted on patients with infrabony defects having minimal or no gingival recession and with a probing depth of >4 mm and controls (12 each). It highlights the use of a newly developed fish collagen–chitosan film as a barrier membrane for the management of human infrabony defects using the principles of GTR in periodontitis subjects. **Results:** Infrabony defects in the test and control sites were matched at baseline. The differences in probing pocket depth within the groups at baseline and 6 months after periodontal therapy were statistically significant. After 6 months, there was statistically significant difference in the probing pocket depth between the test and control sites. **Conclusion:** Within its limitations, the present study showed that the collagen–chitosan film is effective as a barrier membrane for GTR therapy in infrabony defects, as it resulted in improved clinical outcome with low incidence of gingival recession and device exposure.

KEY WORDS: Case–control study, collagen–chitosan barrier membrane, guided tissue regeneration, infrabony defects, periodontal regeneration

INTRODUCTION

Periodontics has been transformed from an empirical mechanical art to a modern clinical discipline based upon established scientific facts. The main objective of traditional periodontal treatment was to resolve the inflammatory lesion in periodontal tissues. But the contemporary goal of periodontal therapy, however, has become regeneration of the lost attachment apparatus. Studies in animals[1,2] and humans[3,4] have demonstrated that it is possible to attain new attachment to the denuded root surfaces by selectively favoring regrowth of periodontal ligament tissue through guided tissue regeneration (GTR) technique. A variety of materials, both non-resorbable and resorbable, have been used in guided periodontal tissue regeneration as barrier membranes. In recent years, an increased trend of use of resorbable barriers for GTR in human infrabony defects has evolved. Generation of biodegradable barrier materials like collagen, polyglycolic acid, polylactic acid, or copolymer materials has been tried. Irrespective of the nature of the material, the GTR membranes currently available in the market are expensive, which in turn precludes the use of such materials for periodontal regeneration. This situation warrants the identification, development, and utilization of more cost-effective, indigenous, and biocompatible materials as barrier membranes for GTR. In this context, the need for the development of a cost-effective barrier membrane for periodontal practice was felt to be essential.

The Central Institute of Fisheries Technology (CIFT), Kochi, Kerala, India, a reputed research institute under the Government of India, developed an indigenous material for biomedical applications. This material consists of a thin film of fish collagen impregnated with chitosan (CHI).

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Address for Correspondence:
Dr. K. Harikumar,
Department of Periodontics, Government Dental College,
Kozhikode, Kerala, India.
E-mail: harisindhu@hotmail.com

Air bladder of freshwater fishes (phylum: Chordata; class: Pisces) which are readily available in this part of the country is the source of collagen. CHI is a natural polymer obtained by the hydrolysis of chitin present on the exoskeleton of shellfish. Shellfishes are also a source of food in this part of the country. This cost-effective, easily available collagen–CHI biomaterial is currently in use after extensive research as "artificial skin" in the field of plastic surgery to cover skin burns in humans in Kerala. Several desirable properties have been described for CHI, including high osteoinductivity, osteointegratability, easy application, and gradual biodegradability, which make it a good candidate for bone regeneration.[5,6] Studies have been done on CHI and its derivatives as materials for the fabrication of scaffolds used for tissue engineering and regeneration.[7,8] The positive results of this pioneering clinical application encourage research on the use of CHI-containing biomaterials for periodontal tissue regeneration. We decided to make use of the properties of this fish collagen–CHI film as a barrier membrane for GTR therapy.

Thus, the present case–control study highlights the use of this newly developed cost-effective, indigenous, and biocompatible fish collagen–CHI barrier membrane for the management of human infrabony defects, based on the principles of GTR. The additional objectives were to compare the results with similar defects treated by conventional open flap debridement, evaluate the clinical and radiographic parameters 6 months postoperatively in both cases and controls, and to evaluate the local soft tissue reactions to collagen–CHI barrier membrane during different stages of healing.

MATERIALS AND METHODS

This study was conducted at the Department of Periodontics, Government Dental College, Thiruvananthapuram, Kerala, India, in collaboration with CIFT. Before beginning the study, clearance of the Institutional Ethics Committee was obtained.

Collagen–CHI barrier membrane

The biomaterial consisting of a thin film of fish collagen, one side of which is impregnated with a layer of CHI, was developed by CIFT. Air bladder of freshwater fishes (phylum: Chordata; class: Pisces) was the source of collagen. A layer of CHI, a polymer derived from the exoskeleton of shellfishes, was impregnated over the processed layer of fish collagen. The material developed by the institute was patented and already being used in the field of plastic surgery as an artificial layer of skin for burn patients.

For periodontal therapy, the biomaterial was fabricated as a barrier membrane suitable for GTR technique. To increase the strength of this barrier membrane and to prolong the *in vivo* persistence, it was cross-linked with 50 ppm glutaraldehyde. It was then sterilized in ethylene oxide chamber and preserved in isopropyl alcohol in sealed packets. The resorption rate following animal subcutaneous tissue implantation was found to be 12 weeks. The material had a tensile strength of 124 kg/cm^2 and a thickness of 0.1 mm. This collagen–CHI barrier membrane was found to be biocompatible and free of any antigenic reactions in preliminary research at the parent institute. For our study, the material was made available in the size of 1 cm × 1 cm sheets. The use of this material in humans was approved by the Human Ethical Committee, Government Medical College, Thiruvananthapuram, Kerala, India.

Patient selection

Age- and sex-matched subjects with periodontitis between the age of 18 and 30 years), except smokers and medically compromised patients, were included in the study. Informed consent was obtained from each patient. Patients with the surgical site having minimal or no gingival recession (GR) at the region of infrabony defects, and with a probing depth of >4 mm were included in the case group (12 patients) and control group (12 patients). Radiographic examination of the surgical site was used to supplement and support the clinical findings. Twenty infrabony defects each were included in the study group and in the control group. Conventional open flap debridement alone was planned in selected infrabony defects in the control group. Similar defects to be treated with collagen–CHI barrier membrane using the principles of GTR formed the study group.

Initial/cause-related phase

Prior to surgery, each patient was given careful instructions on proper oral hygiene techniques. A full-mouth supragingival and subgingival scaling and root planing was performed. All clinical parameters at baseline were recorded. Preoperative periapical radiographs of each patient, standardized with long-cone technique and film positioners, and ruled grids were kept for radiographic assessment. Probing pocket depth (PPD) was taken as the distance from the gingival margin to the base of the pocket. Gingival margin level (GML) was recorded as the distance from the cemento-enamel (CE) junction to the crest of the gingival margin, and probing attachment level (PAL) was calculated as the distance from the CE junction to the base of the pocket (PPD - GML). All measurements were made at four aspects of the tooth and corrected to the nearest millimeter [Figure 1a and b].

Surgical phase

Surgical procedure in selected infrabony defects was done 3 months after the initial/cause-related phase. Following

local anesthesia, intracrevicular incisions were made and full-thickness flaps were raised at the buccal and lingual/palatal aspects of the experimental tooth. The flap was extended mesially and distally, so as to get optimum access to the defect. All granulation tissue was removed and the inner surface of the flap was carefully trimmed to remove the pocket epithelium. No osseous recontouring was done. The root surfaces were scaled and planed to remove all subgingival soft and hard deposits. The actual periodontal attachment loss in each defect was recorded after surgical exposure and debridement [Figure 2]. The collagen–CHI membrane was soaked in distilled water in order to remove excess alcohol to improve adhesion properties. The membrane was trimmed into the suitable configuration so as to cover the defect. It was then adapted over the defect, extending 2-3 mm apical to the crest of the existing bone, so as to provide a broad base during placement, with the CHI side (the rough surface of the membrane) contacting the soft tissue portion [Figure 3a and b]. The coronal portion was tightly secured to the CE junction of the tooth. The flaps were secured with interdental sutures to obtain primary closure of the interdental tissues over the membrane. A non-eugenol dressing was given at the surgical site. Sutures were removed after 7-10 days. In the control sites, following open flap debridement, the flap was sutured back to the tooth without placing the collagen–CHI barrier device over the defects.

Post-surgical care

The patients were advised to rinse with a 0.12% solution of chlorhexidine gluconate twice a day for 6 weeks following surgery. Analgesics were prescribed when indicated. In addition, all these patients received systemic antibiotic therapy (ciprofloxacin 500 mg + tinidazole 300 mg) twice daily for a period of 5 days, starting 1 h before surgery. All patients were instructed not to brush the surgical site until the sutures are removed. Mechanical tooth brushing including interdental brushing in the surgical area was reinstituted following the removal of sutures. All patients were recalled for professional tooth cleaning once every 2 weeks for the first 3 months following surgery and once every 4 weeks for the next 3 months. After 6 months of healing, the patients were enrolled in a maintenance program with recall intervals according to individual needs.

Statistical analysis

All data were expressed as means and standard deviation (SD) of the infrabony defects (20 each in the test and control groups). Non-parametric Wilcoxon matched pairs signed-rank test and Wilcoxon rank-sum test were used to assess the statistical significance between the different parameters at baseline and 6 months postoperatively, and between the postoperative results of the test and control groups. Statistical significance was declared if the *P* value was found to be ≤ 0.05.

RESULTS

Infrabony defects in the test and control sites were matched at baseline [Table 1]. The difference in PPD within the groups at baseline and 6 months after the periodontal therapy was statistically significant. After

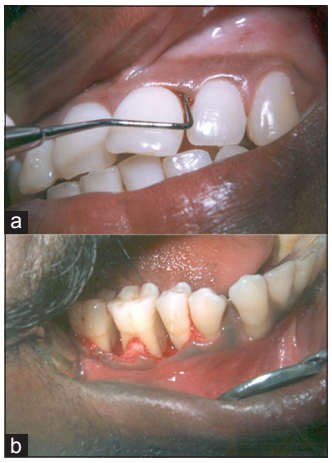

Figure 1: (a) Pre-op infrabony defect; (b) pre-op molar infrabony defect

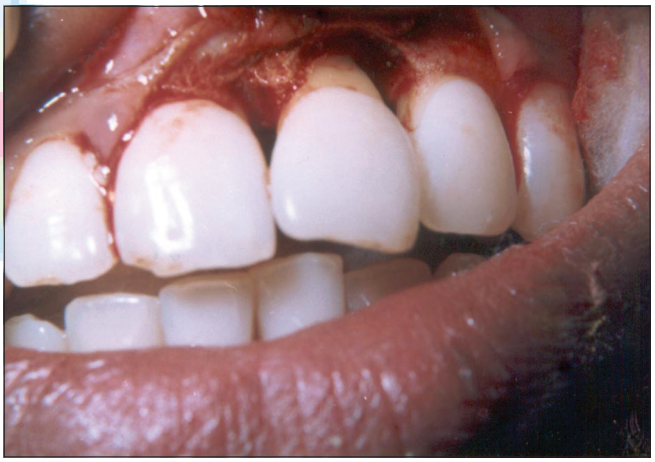

Figure 2: Post exposure bony defect

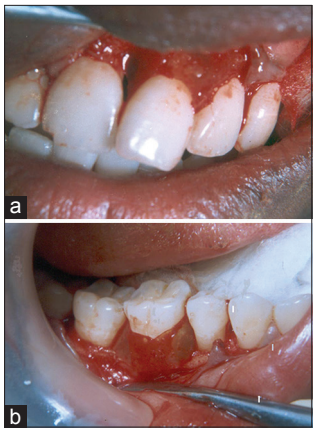

Figure 3: (a) Infrabony defect with collagen–chitosan; (b) bony defect with collagen–chitosan

6 months, there was statistically significant difference in the PPD between the test and control sites [Table 1]. The mean PAL gain in the test sites 6 months after the periodontal therapy was 3.8 ± 1.3219 mm and was statistically significant [Table 1]. Although the groups did not differ in PAL at baseline, there was a statistically significant difference between the groups 6 months postoperatively [Table 1 and Figure 4].

Postoperative intraoral periapical radiographs at 6 months were taken to compare the bone fill with the preoperative radiographs [Figures 5 and 6]. Radiographic evidence of bone formation was evident in the postoperative radiographs. Although it is considered that radiographic evidence of bone formation will be evident 9 months following regenerative therapy, we have taken radiographs only after 6 months as per our study design. Histologic evaluation is the gold standard in demonstrating new attachment; but it was not possible in this study due to ethical reasons. Thus, we were forced to interpret the results with our statistically significant clinical and radiographic findings 6 months following the procedure.

DISCUSSION

The material used for achieving periodontal regeneration should be biocompatible, cost-effective, and easy to apply. The present study evaluates the effectiveness of an indigenous preparation of a collagen–CHI film, a resorbable barrier membrane, to obtain GTR in infrabony defects in humans. The development of a cost-effective, indigenous, and biocompatible material as barrier membrane for GTR seems highly essential for periodontal practice in our part of the world India. Both collagen and CHI, chosen for the barrier membrane preparation in the present study, have been extracted from readily available local food sources. Resorbable collagen barrier membranes are already available for GTR therapy and are time tested[9] CHI is a relatively new entry into dental practice. It has been reported as a biodegradable and biocompatible substance.[10] Several studies have investigated various effects of CHI on bone healing and have proposed some hypotheses on its mechanisms.[10,11] CHI increases the vascularization with the formation of new blood vessels,[12] activates osteoblasts thereby increasing osteogenesis,[13] facilitates wound healing,[14] and has antimicrobial properties, and hence used in orthopedic procedures and craniofacial implants.

Table 1: Comparison of clinical parameters at baseline and 6 months postoperatively within and between groups

Parameter	Test site mean (SD)		Control site mean (SD)	
	Baseline	6 months postoperative	Baseline	6 months postoperative
Probing pocket depth	6.7 (1.559)	2.5 (0.510)[a,b]	6.7 (1.559)	3.5 (0.826)[a,b]
Probing attachment level	5.8 (0.5)	3.8 (1.3219)[a,b]	5.5 (0.3)	2.1 (0.516)[a,b]

[a]The difference within the group at baseline and 6 months after periodontal therapy was statistically significant. [b]After 6 months, there was statistically significant difference between the groups. SD = Standard deviation

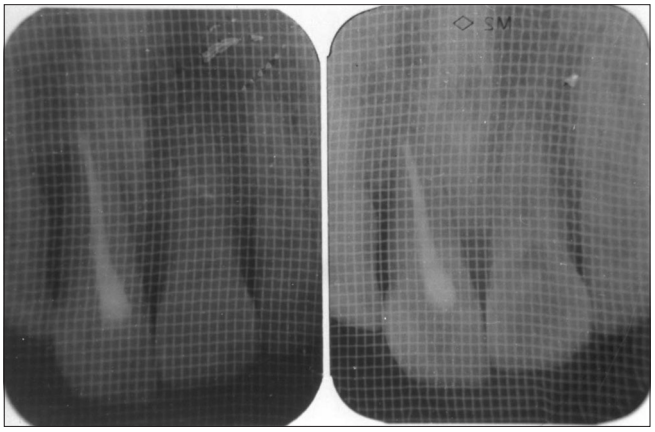

Figure 4: Pre and post xrays showing bone level changes in the incisor area

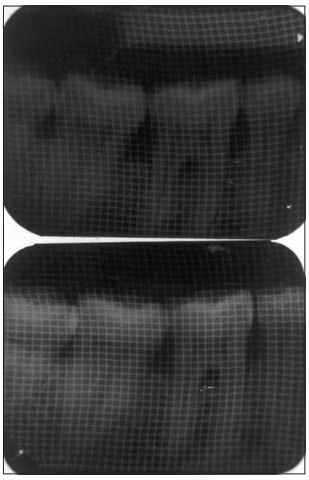

Figure 5: Pre and post xrays showing bone level changes in the molar area

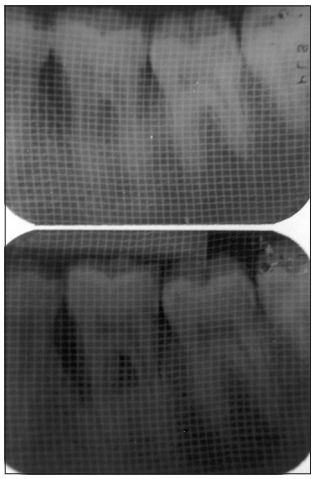

Figure 6: Preoperative and postoperative radiographs - molar region

In the present study, periodontal infrabony defects managed by GTR using collagen–CHI as a barrier membrane were evaluated over a time interval of 6 months. Previous studies have shown that dimensional changes of periodontal tissues mostly occur within the first 6 months following conventional surgery[15] and also from GTR procedures.[16] Mangnuson *et al*.[17] assessed different time points during healing and interpreted that the critical phase of healing occurred within the first 2 months following GTR therapy. In this study, mean pocket depth reduction in the test group was more than in the control group and was statistically significant, which concurs with the results reported by Falk *et al*.[18]

According to Iglhaut *et al*.,[19] the factors that seem to enable periodontal regeneration include the morphology of the defect with good support of the membrane on the residual bony walls of the defect preventing its collapse, a large surface area of both periodontal ligament and alveolar bone adjacent to the blood clot enabling repopulation of the wound by progenitor cells, and the availability of adequate nutrient supply necessary for wound healing process. In our study, the membrane was placed over the defects, so that the apical portion extended and rested on a broader area of the remaining alveolar bone. This might have prevented collapse of the membrane into the defects in the case of infrabony sites.

Also, in our study, infrabony defects of similar probing depth demonstrated different levels of probing depth reduction and attachment gain. This can be attributed to the lack of similarity in the intrabony component of these defects. Benqué *et al*.[20] showed that a deep, three-wall intrabony defect has a different potential for regeneration than two-wall intrabony defects. They also noticed disparity in the attachment gain in two- and three-wall intrabony defects treated using the principle of GTR.

Cortelleni *et al*.[21] evaluated osseous healing following GTR and indicated that healing of infrabony defects represented a combination of marginal crest resorption and apical bone fill. However, it has been suggested that the usual 6- to 12-month reentry procedure may be too early because of the continuous maturation of the osseous graft.[22] Research has demonstrated that osteoid may not be completely formed by 8 months and that the maturation process of bone may take up to 2 years.[22] Another disadvantage of using reentry procedures in clinical research is that the recruitment of study patients and approval from institutional ethics committee may be more difficult than less-invasive means of outcome assessment. In our study, intraoral periapical radiographic evaluation of the test sites showed more "bone fill" than the control sites, as evident in the postoperative radiographs.

In the present study, 4 out of 20 infrabony test sites (20%) showed GR postoperatively. Exposure of the membrane occurred at the same sites. Although the coronal extent of the membrane was limited to the CE junction during placement, the shrinkage of the flap after surgery may be the cause of the device exposure. Selvig *et al*.[2] reported in their study that to reduce recession, to maintain flap position, and for the ease of patients' oral hygiene, subgingival placement of the barrier membrane is essential. They also noted that recession was impeded when crown-attached sutures were used to hold flaps coronally. This technique can be incorporated into the surgical technique for GTR with resorbable barriers. The recession observed in four of the test sites did not seem to increase with time. This was evidenced by the maintenance of the clinical attachment gain even after 6 months of surgery, since it has been shown that new attachment formation is negatively influenced by GR and infection.

One of the pivotal issues concerning GTR is how long the barrier should stay in place. The collagen–CHI membrane used in this study is considered to resorb completely within 12 weeks as per the animal subcutaneous implantation done in the parent institute. Benque *et al*.[20] proposed a time of 4-6 weeks for the membrane to stay in place. This was based on the report of Iglhaut *et al*.,[19] which states that the amplifying divisions of periodontal ligament cells may be completed in 21 days. Pitaru *et al*.[23] in their study found that if collagen barriers are resorbed before day 30, new cementum can be found in the area, but no new bone. However, it was found that 10 days duration is long enough to prevent the apical migration of junctional epithelium. The collagen–CHI membrane that was found exposed over the gingival margin in the infrabony test sites postoperatively disappeared completely in 6-8 weeks time as observed clinically. So, it can be assumed that collagen–CHI has got an *in vivo* persistence as needed to ensure GTR in periodontal defects.[24]

Moreover, the collagen–CHI membrane was well tolerated at various experimental time intervals without any untoward inflammatory reactions. This excellent biocompatibility observed confirmed the results already obtained in animal studies after subcutaneous implantation of this material in the parent institute.

A drawback of collagen membranes is their tendency to collapse into the osseous defect if not supported or reinforced. Collagen–CHI membrane used in this study is cross-linked with 50 ppm glutaraldehyde to increase the strength, stiffness, and to some extent, the *in vivo* persistence of the device. Quiteish *et al*.[25] used glutaraldehyde cross-linked collagen membrane for GTR in humans and got statistically significant gain in attachment in the test group.

In this 6-month clinical trial of GTR using collagen–CHI barrier device, the results were evaluated by clinical and radiographic means. Although the postoperative radiographs taken 6 months after the procedure revealed some amount of bone fill, further radiographic evaluation of test sites at 9 months and 1 year is essential to provide substantial evidence of bone fill in comparison with preoperative radiographs. This can be a limitation of this study. Histologic evaluation and surgical reentry, which confirms "new attachment" formation, were not attempted due to ethical reasons. Until long-term follow-up is available, it may be prudent to maintain a conservative perspective toward the real clinical effectiveness of this GTR procedure. So, further studies are needed to evaluate the long-term results of this study using collagen–CHI film as the barrier membrane for GTR.

CONCLUSION

Since research on bioresorbable membranes is in constant progress, it is often difficult to compare the results of one product with an identical product. Within its limitations, the present case–control study showed that the use of collagen–CHI barrier for GTR therapy in infrabony defects resulted in improved clinical outcome with low incidence of GR and device exposure. It was not associated with any local or systemic adverse reactions or clinically detectable allergic reactions. This indigenous collagen–CHI membrane has got excellent handling characteristics, ease of placement, and biologic acceptance to be used for GTR technique. With this being a 6-month clinical study, further controlled clinical trials on a long-term basis are needed to confirm the effectiveness of collagen–CHI membrane in periodontal GTR therapy.

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