Management of periodontal furcation defects by guided tissue regeneration using collagen – chitosan as a barrier membrane

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Abstract
Guided Tissue Regeneration (GTR) is treatment modalities that bring out the ideal healing in the periodontal wound in the form of New Attachment. A lot of resorbable and nonresorbable barrier membranes are used for this procedure. In this study we used a novel, indigenous and economic material as barrier membrane for GTR. When this material is used to treat selected furcation defects, statistically significant improvements in clinical paramaters were observed. Untoward reactions in any form were noted in any of the clinical sites.

Introduction
Our knowledge and the practice of clinical periodontology have increased many folds since Periodontics has organized as a dental specialty in 1914. Now, Periodontics has entered into a plateau of maturity and into a greater sense of security than has been the case previously. 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 the regeneration of the lost attachment apparatus and its treatment. Studies in animals and humans have demonstrated that it is possible to attain new connective tissue attachment (New Attachment) to the denuded root surfaces by selectively favoring regrowth of periodontal ligament tissue.[2] This technique, Guided Tissue Regeneration (GIR) is accomplished by placing a barrier device between the gingiva and root surface there by excluding unwanted tissues from reaching contact with the root during healing. This concept of GTR was proposed by Melcher in 1976. The present study highlights the use of a newly developed economical resorbable fish collagen barrier - a Collagen - Chitosan Film - for the management of human infrabony and furcation defects using the principles of GTR. This material is developed by the Central Institute of Fisheries Technology (CIFT) - Cochin.

Aims and Objectives
1. To use collagen - Chitosan as a barrier device for GTR in human periodontal class II furcation defects.
2. To compare these results with similar defects treated by conventional open flap debridement.
3. Evaluation of clinical parameters 6 months post operatively in both study group and control group.
4. To evaluate local soft tissue reactions to collagen – chitosan during different stages of hea1ing.

Materials and methods: Collagen: Chitosan Barrier Material
This is a resorbable membrane originally developed as "Synthetic skin" to cover burn wounds and is now used in the Department of Plastic Surgery, Govt. Medical College, Kozhikode. Material is developed by Central Institute of Fisheries Technology, Cochin; This material consists of a thin film of fish collagen one side of which is impregnated with a layer of chitosan.

Collagen: The source of collagen is from the airbladder of fresh water fishes (phylum - chordata - class -Pisces. eg:Sacchobronchus, Ophiocephalus etc) These group of fishes are used as food in many parts of our country. The air bladder in them is an accessory respiratory organ used for areal respiration.

Chitosan: Chitosan used in this barrier material is a hydrophobic biopolymer obtained by hydrolyzing aniinoacetyl groups of chitin, a protein in the shells of crab and shrimp, by alkaline treatment. Chitosan is widely used in the field of Biomedical, Cosmetics, Pharmaceuticals, Food products, Agricultural materials etc. Its chemical formula is $C_6H_{11}O_4N_3$. It has found biocompatible and free of any antigenic reactions. is sterilized in Ethylene Oxide chamber and preserved in isopropyl alcohol in sealed packets. To increase the strength to prolong the in vivo persistence it is crosslinked with glutaraldehyde -50 ppm. The resorption rate following animal subcutaneous tissue implantation is about 12 weeks. The material is having a tensile strength of 124 Kg/cm2 and a thickness of 0.1 mm. For this study, the material was made available in the size of 1 cm x 1 cm sheets.

The use of this material in humans is approved by the Human Ethical Committee, Medical College Thiruvananthapuram and Kozhikode.

The patients reporting to the post graduate clinic, Department of Periodontia, Government Dental College Thiruvananthapuram were selected for the study. The study group and the control group consist of both males

and females with chronic periodontitis between the ages of 18-30 years. Conventional open flap debridement was done in selected infrabony and furcation defects in the control group. Similar defects treated with collagen - chitosan barrier membrane using the principles of GTR formed the study group

Patient Selection

1. Patients, referred for specialist treatment of advanced periodontal disease were included in this study on a voluntary basis.
2. All patients participating in the study were given an information sheet and a consent was obtained from each of them.
3. Clinically determined Grade II furcation defects on mandibular I Molars with a horizontal probing depth not exceeding 8mm were included in the study.
4. The surgical site should have minimal or no gingival recession
5. The patient should be able to participate in a 6 month clinical trial.
6. All patient should be non-smokers and medically fit for the procedure.

Fifteen furcation defects were treated in the study group and fifteen similar defects were treated in the control group.

The necessary patient details including all clinical parameters at Baseline, and 6 months post-operative were recorded in the proforma.

Pre Surgical Care

Prior to surgery, each patient was given careful instructions on proper oral hygeine techniques. A full mouth supra and subgingival scaling and root planning was performed. Only patients who, following the hygienic treatment phase, documented willingness and ability to maintain optimal oral hygiene were included in the study

Surgical Procedure

Following Local Anaesthesia (Lignocaine - Adrenaline), Intracrevicular incisions were made and full thickness flaps raised at the buccal and lingual/palatal aspects of experimental tooth. All granulation tissue was removed and the inner surface of the flap was carefully trimmed to remove the dentogingival epithelium. The root surfaces were scaled and planned to remove all subgingival soft and hard deposits and the area is rinsed with sterile saline. Root conditioning is done using tetracycline hydrochloride (100mg! ml) for 4 minutes. Following debridement, collagen-chitosan membrane is trimmed into the suitable configuration so as to cover the defect. The membrane is to be soaked in distilled water before this, in order to remove excess alcohol arid improve adhesion properties. It is then adapted over the defect extending 2-3mm apical to the crest of the existing bone so as to provide a broad base during placement, the chitosan side- the rough surface, of the membrane should contact the soft tissue portion. The coronal portion of collagen chitosan is tightly secured to the cement enamel junction of the tooth with preplaced, bioresorbable 4-0 catgut ligatures. The flaps were secured with interdental sutures to obtain primary closure of the interdental tissues over the membrane. A Zinc Oxide Eugenol dressing was given at the surgical site. Sutures were removed after 7-10 days

In the control group, following open flap debridement, the flap is sutured back to the tooth without placing collagen-chitosan barrier device over the defects.

Post-Surgical Care

The patients were advised to rinse with a 0.12% solution of chiorhexidine gluconate twice a day for 6 weeks following surgery. Analgesics were prescribed when indicated. In addition all these patients received systemic antibiotic therapy (Ciprofloxacin 500mg + Tinidazole 300mg) twice daily for a period of 5 days, starting 1 hr before surgery) Mechanical tooth cleaning in the surgical area was reinstituted following the removal of sutures.

Assessments

Clinical measurements were made by the same individual during each visit. The following variables were recorded prior to surgery (baseline), at the time of surgery, 1½ months, 3 months and 6 months after surgery. All measurements were done by the same Williams graduated probe and Naber'sprobe.

1. Probing Pocket Depth (PPD) - The distance from the gingival margin to the base of pocket.
2. Gingival Margin Level (GML) - The distance from the CE junction to the crest of gingiva.
3. Probing Attachment Level (PAL) - The distance from the CE. Junction to the base of the pocket. (PPD - CML)
4. Horizontal Probing Depth at Furcation (PAL -H) - Assessment made at the roof of the furcation area.
5. Vertical Probing Depth at Furcation (PAL -V)

All Measurements were made at 4 aspects of the tooth except furcations and corrected to the nearest millimeter. Plaque Index (Silness and Loe) was also recorded during the follow up visits.

Data were expressed as means and standard deviation (SD) of twenty six test and twenty six control sites. Statistical Analysis were done for infrabony and furcation defects separately. The changes over time of clinical parameters (Probing Pocket Depth - PPD, Gingival Margin Level -CML and Probing Attachment Level -PAL) were recorded in millimeters and the attachment gain calculated- the pairs being made by measurements at baseline and six months post operatively for each defect and parameter in the test and control group. All statistical analysis were computerized and using the normal probability plot, it was found that none of the clinical parameter followed the distribution normality. Therefore we applied non - parametric tests- "Wilcoxon matched pairs signed rank

test" and "The Wilcoxon rank sum test" to assess the statistical significance between the different parameters at baseline and six months post operatively and between the post-operative results of test and control group. Statistical significance was declared if the" P -value" was found less than or equal to 0.05. Results of data collected and statistical analysis are given in the tables I, II, III, IV.

Statistical analysis was done between the baseline and 6 months postoperative values.

Clinical Results

Furcation defects: In the test group horizontal probing depth of furcation PAL–H, changed from 5.83 ± 1.835mm to 2.83 ± 0.408 mm with a mean PAL gain of 3 ± 0.981mm. (P = 00277). In the control group horizontal probing depth of furcation changed from 5.83 ± 1.835mm to 3.66 ± 0.516mm with the mean PAL gain of 217 ± 0,668mm (P= 0.0431) . Vertical probing depth of furcation PAL- V, in the test group changed from 4.5 ± 1.378mm to 1.83 ± 0.753mm, the mean PAL gain being 2.67 ± 0.828mm (P= 0.0431). In the control group the vertical probing depth of furcation changed from 4.5 ± 1.378mmto 2.5 ± 0.826mm, the PAL gain being 2 ± O.535nmt (P= 0.0679).

In the case of furcation defects a definite reduction in the horizontal and vertical probing depth of furcations indicating a gain in clinical attachment following surgery was noticed. When the post-operative results were analysed, the results were not statistically significant in case of attachment gain in vertical depth of furcation (PAL-H-P = 0.0411; PAL-V P 0.1797).)

The mean values of pocket depth reduction and attachment gain in furcation defects of test and control group dining baseline 1 .5months, 3 months and 6 months are plotted in the form of bar diagram and are shown below. (Table 1,2,3)

Table: I Furcation defects change in horizontal probing depth of furcation (PAL-H)

PPD Variable	Mean in MM			
	Test	SD	Control	SD
Baseline	5.8	1.835	5.8	1.835
1.5 month	3.0		4.2	
3 month	2.8		3.6	
6 month	2.8	0.408	3.6	0.516
P value	**0.0277		**0.0001	

* Statistically significant

Table: II Furcation defects change in vertical probing depth of furcation (PAL-V)

PPD Variable	Mean in MM			
	Test	SD	Control	SD
Baseline	4.5	1.378	4.5	1.378
1.5 month	2.8		2.8	
3 month	1.8		2.5	
6 month	1.8	0.753	2.5	0.826
P value	*0.0431		0.0679	

Table: III Clinical Attachment

	Mean in MM				P value
6 months post op	Test	SD	Control	SD	
Infra bony	3.8	±1.3219	3.1	±0.965	**0.0001
Furcation PAL-H	3.0	± 0.981	2.17	±0.668	*0.0411
Furcation PAL-V	2.67	±0.828	2.0	±0.535	0.1797

* Statistically significant ** highly statistically significant

Discussion

The present study evaluates the effectiveness of this collagen-chitosan film, a resorbable barrier membrane, to obtain guided tissue regeneration in furcation defects in humans.

In the case of furcation defects horizontal probing depth of furcation involvement (PAL-H) showed clinically and statistically significant reduction in the test group (P = 0.0277) and in the control group (P = 0.0431). The attachment gain in the test group was found more and statistically significant (P = 0.0411) when compared with the control group. Also, three of the six treated sites (50%) in the test group showed a change from grade II furcation to grade I furcation. None of the defects were complete1y closed. The change in the grade of furcation obtained in the present study reflects the reduction of horizontal interradicular probe penetration. The reason for this change can be due to the formation of a new connective tissue attachment or a long junctional epithelium between the root surface and the newly formed dense connective tissue[18] Similar comparable results are obtained in previous studies by Becker et al 1988 arid Pontoriero et al 1988.[17]

On the other hand the vertical probing depth in the furcation area (PAL -V) showed clinically and statistically significant reduction in the test group (P 0.0431) and statistically insignificant reduction in the control group (P = 0.0679). The post-operative attachment gain between the test and control group were not statistically significant (P = 0.1797), allthough the test sites showed slightly better gain in clinical attachment. One of the reasons for this may be due to the collapse of collagen-chitosan membrane to the base of the defect. In the present study, four out of twenty infrabony test sites (20%) showed gingival recession

post operatively. Exposure of the membrane occurred at the same sites.. Displacement of the membrane can also result in device exposure .

In this study, tetracycline hydrochloride is used for root conditioning. It is used in a concentration of 100 mg/ml (10%) and applied for four minutes. Trombelli et al (1995)[6] have shown that a four minute application of 10% tetracycline hydrochloride on a planed root surface exposed a network of peritubular and intertubular collagen fibrils enhancing fibroblast attachment and growth.

Summary and Concussions

1. Probing pocket depth reduction in furcation sites with collagen- chitosan was more and statistically significant than control sites.
2. Clinically significant attachment gain was noticed in the horizontal probing depth of furcation involvement in both groups which were statistically significant.
3. The amount of horizontal gain in the furcation sites with collagen-chitosan was more and statistically significant than control sites
4. Clinically significant attachment gain was noticed in the vertical probing depth of furcation only in the collagen - chitosan treated sites. This gain when compared with the control was found statistically insignificant.
5. The use of collagen-chitosan for GTR resulted in low incidence of gingival recession, device exposure and gingival pathology.
6. The use of collagen-chitosan is not associated with any local or systemic adverse reactions or clinically detectable allergic reactions.
7. Collagen-chitosan has got excellent handling characteristics, ease of placement and biologic acceptance to be used as a barrier device for GTR.

Since research on bioresorbable membranes is in constant progress, it is often difficult to compare results with an identical product. The present case-control study showed that the use of collagen-chitosan barrier for GTR therapy in infrabony and furcation defects resulted in significantly improved clinical and radiographic results. This being a six month clinical study, further controlled clinical trials on a long term basis are needed to confirm the effectiveness of collagen-chitosan membrane in periodontal guided tissue regeneration therapy

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