Antibacterial efficacy of Collagen membrane: an Invitro Study

Dr Pooja Walia[1], Dr. Sweta Yadav[2]

Associate Professor[1], Senior Lecturer[2]
Dept of Periodontology, Teerthanker Mahaveer Dental College and Research Centre, Delhi Road, Moradabad (UP)[12]

Abstract:
Introduction: GTR procedures have been used successfully used to treat various types of intrabony defects. Various barrier membranes are used in guided tissue and bone regeneration. One of the main reasons for unsuccessful regenerative outcomes is the colonization and penetration of bacteria through barrier membrane. Certain periodontal pathogens show high adherence to GTR membranses. The antibacterial activity in GTR membranes can control inflammation and collagenolytic activity of bacteria, thus, improving the outcome. **Aim**: The aim of the present study was to evaluate the anti bacterial properties of two different resorbable collagen membranes. **Material and methods:** Two collagen membranes were tested against the bacterial strains (*A. actinomycetemcomitans, and P. gingivalis*) using direct contact test. The optical densities of bacterial growth were evaluated using Spectrophotometer. **Results:** The presence of the membrane samples did not disrupt bacterial growth. However, both the membranes accelerated bacterial growth rate of *A. actinomycetemcomitans, and P. gingivalis* as compared to the control samples ($P < .001$). **Conclusion:** Collagen membrane enhances the growth of periopathogens in vitro and maybe a potential risk to regeneration.

Keywords: **Keywords:** Periodontitis, Collagen, Membranes, Regeneration, Resorbable

INTRODUCTION

Guided tissue regeneration(GTR) is the most commonly used method to augment bone volume and induce new tissue growth. GTR procedures have been used successfully used to treat intrabony defects causing pocket elimination, clinical attachment gain, and alveolar bone gain. [1] Regenerative periodontal therapy restores the periodontal tissues (i.e. new periodontal ligament, new cementum with periodontal ligament fibres and new alveolar bone formation) that have been lost due to periodontal disease. Placement of barrier membranes against the bony

Correspondence Address: Dr. Pooja Walia, Department of Periodontology, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India.
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defect prevents the gingival epithelium and connective tissue to migrate to the root surface. Moreover, it allows migration of the PDL cells into the defect which helps in regeneration of the tissues.[2]

However, there are several factors such as plaque control, periodontal infection, and smoking habits of the patient that influence the success of GTR. [3]

Barrier membranes are divided into two main groups, resorbable and non – resorbable barrier membrane.[4] These membranes as demonstrated, because of the nature of their surface and adsorption of organic material from the serum or the saliva present a interface for colonizing cells [5]

Several studies have suggested that optimal tissue regeneration cannot be expected for barrier membranes placed in sites infected by periodontopathic microorganisms. Hence, a prerequisite for successful regeneration is an infection-free healing process.[6]

Certain periodontopathic bacteria show high adherence to collagen membrane. The proteolytic activity of these bacteria may cause membrane degradation rapidly and alter the regenerative process.[7,8] The antibacterial activity of the collagen membrane can therefore, control the inflammatory reaction and thus cause an enhancement in the regenerative process.

Hence, the aim of the present study was to assess the antibacterial properties of two different cross-linked resorbable collagen membranes.

MATERIALS & METHODS

The study was conducted in KLE V.K. Institute of Dental sciences, Belgaum.

Porphyromonas gingivalis and A. actinomycetemcomitans were cultured in the laboratory.

A. actinomycetemcomitans was cultured on an anaerobic blood agar plate and incubated at 35˚C in an anaerobic chamber with anaerobic gas mixture.

The membranes cologuide and periocol were tested for antibacterial properties against, porphyromonas gingivalis and A. actinomycetemcomitans using direct contact test. Samples of each membrane sized 5x 2 mm were fixed to the side wall of six wells in a 96 well microtitre plate. Bacterial suspensions were placed on each sample and incubated at 37˚C for 1 hour. Non contaminated membrane sample served as negative control. Bacterial growth was assessed in a spectrophotometer at 650nm for 24 hours.

The Direct contact test(DCT), was observed in each well every 30 min for 16 hours using a spectrophotometer at 600nm at 37◦C. 96 wells of a microtitre plate were used out of which 8 wells were utilized.

Out of these 4 were designated as “A” wells and the other 4 as “B” wells (Fig 1).

This assay is based on causing bacteria to come in close contact with the tested material. Later on, assessment of microbial growth was done.

Six samples of each collagen membrane, sized 5 × 2 mm^2, were fixed to the wall of six wells (group A). Bacterial suspension (10 μL) was placed on the surface of each collagen membrane. The plate was then placed into an incubator for 1 hour at 37°C, for evaporation of the bacterial suspension fluids, so that there is direct contact between the bacteria and the collagen membranes(CM). After 1 hour, BHI broth was added to the Group A wells and gently stirred for 1-2 minutes. Then 10 μL of suspension were transferred from Group A wells to an adjoining set of six wells (Group B). This resulted in two sets of six wells for each membrane containing an equal amount of the medium to monitor the bacterial growth, both in the presence and absence of the test(collagen) membrane. One set of the wells (three wells) in the microtiter plate was designated as positive control.

The growth curves from test groups were compared with the control groups. The negative control comprised of three wells with the test membranes similar to experimental Group A, containing an identical volume of the media. The growth in each well was monitored at 650 nm at 37°C and recorded every 30 minutes for 24 hours, using the same temperature-controlled spectrophotometer (Fig 2). Data were measured in optical density (OD units).

The values of Optical density(OD) from the negative control were considered baseline and these were subtracted from the experimental data. These were then displayed as growth curves.

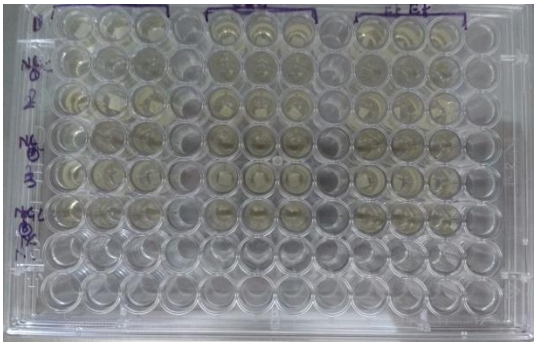
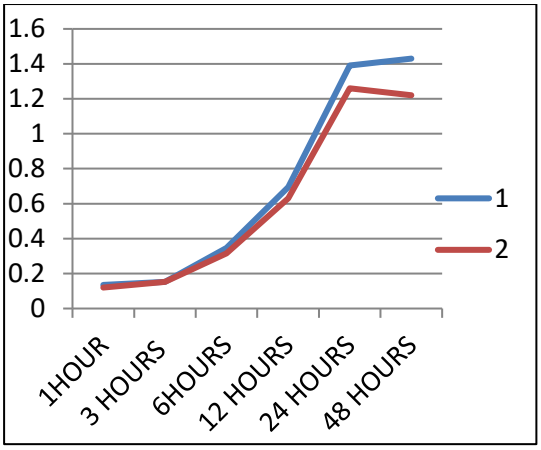
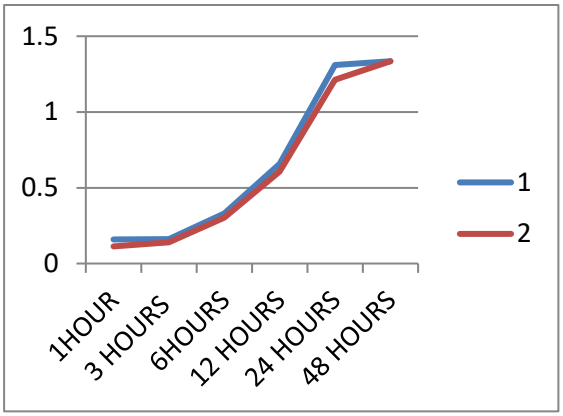


Fig 1. 96 wells microtitre plate

Fig 2. Spectrophotometer



Graph 1. P gingivalis growth



Graph 2. A. Actinomycetemcomitans growth

STATISTICAL ANALYSIS

SPSS version 21 was used for statistical analysis. Analysis of variance (ANOVA) was applied to compare the growth rate of bacteria.

RESULT

Porphyromonas gingivalis

The collagen membranes - healiguide and cologuide in the suspension did not reduce the growth of Porphyromonas gingivalis in comparison to the control group. However, it was found that these membranes accelerated the bacterial growth of Porphyromonas gingivalis in comparison to the control group ($P < .001$). (Graph 1)

Aggregatibacter actinomycetemcomitans

The collagen membrane - healiguide and cologuide did not reduce the growth of Porphyromonas gingivalis in comparison to the control group. However, it was found that both the membranes accelerated the growth rate of A. actinomycetemcomitans_as compared to the control group ($P < .001$). (Graph 2)

DISCUSSION

Collagen is a major component of the membranes. The bacteria that colonize these collagen membranes were found to degrade collagen by their proteolytic activity.[9] As these bacteria pass through the membranes along with fibroblasts and giant cells or invade these membranes and colonize its surface. Higher tendency of bacteria to adhere to these membranes is due to their hydrophilic property.

We found that the membranes did not reduce the growth of the bacteria in comparison to the control groups i.e both the membranes had no antibacterial properties. However, it was found that the

collagen membranes presented an ability to increase the bacterial growth.[10,11]

It can be stated that the two collagen membranes used in this study had more hydrophilic property due to their crosslinked structure. Thus, we found that there was an increased growth of P. gingivalis and A. actinomycetemcomitans as compared to control, however, this difference was not statistically significant.[12]

Wang et al. evaluated the initial adhesion of a variety of bacteria to three barrier membranes(expanded polytetrafluoroethylene [e-PTFE], polyglactin, and collagen) and found patterns of selective adhesion to the different membranes.[6] Studies by Sela et al showed that certain bacteria have high affinity to collagen membranes.[7]

Similarly, Chogle and Mickel in their study observed a delay in bacterial growth on a polylactic acid (PLA) membrane compared to two other BM. They attributed the membrane's antibacterial effect to the high hydrophobicity of PLA.[13]

The Direct Contact Test (DCT) used in this study is a routinely used method for evaluating antibacterial activities.

Unsuccessful regenerative procedures (GTR), is most commonly due to the colonization and penetration of these periodontopathic bacteria through the collagen membranes into the surgical site. Various studies that examined the outcome of regeneration in the presence of bacteria found that the membranes used for periodontal regenerative procedures were frequently colonized by periodontal bacteria.

A study conducted by Slutzkey et al (2015), tested 3 collagen membranes-BioGuide (non cross-linked), OsseoGuard (cross-linked) and CopiOs (non cross-linked). His study found that OsseoGuard which was cross-linked possess no antibacterial properties, moreover, they showed increased bacterial growth. The bacteria colonizing the membranes were found to have the ability to degrade collagen membranes by proteolytic activity.[15] These bacterial properties explain the correlation found between the presence of bacteria in e-PTFE and polyglycolactic membranes and reduction in gain of attachment. Additionally, an association was found between periodontal pathogens and unsuccessful regenerative procedures.

Therefore, due to the adverse influence that bacterial colonization of BM has on regenerative procedures, the present findings suggest that the membrane increases bacterial growth and thus, may have clinical implications in various regenerative procedures.

CONCLUSION

The key to any successful regeneration is to provide an environment which prevents the growth of bacteria. The collagen membranes tested have no antibacterial properties. Moreover, the membranes showed a increased bacterial growth. These findings suggest the clinician to meticulous use of these membranes as they may influence the outcome of GTR procedures.

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