Antibacterial Efficacy of Collagen and Foetal Barrier Membranes

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ABSTRACT

Introduction: A wide range of barrier membranes are used in guided tissue and bone regeneration for reconstructing lost periodontium. One of the main reasons for unsuccessful regenerative procedures is the colonization and penetration of bacteria through barrier membrane into the treated site. Certain bacteria have high adherence to GTR membranes with periodontal pathogens showing greater affinity. Antibacterial properties of amniotic fluid are well documented and demonstrated. No such factors have yet been found in amniotic membranes. The antibacterial activity if present in GTR membranes can control inflammatory lesion and collagenolytic activity of bacteria increasing the life of the membrane and treatment outcome. Study aimed to investigate the antibacterial properties of collagen and foetal barrier membranes.

Material and methods: Three membranes (Collagen, Chorion, Amnion) were tested against three bacterial strains (*S. aureus, A. actinomycetemcomitans, P. gingivalis*) using direct contact test. The optical densities of bacterial growth were evaluated using Spectrophotometry.

Results: There was no statistical significant difference in the growth of bacteria for Amnion and Collagen membrane. However there is a statistically significant increased growth of P. gingivalis and A. actinomycetemcomitans on Chorion membrane.

Conclusions: Thus chorion membrane enhances the growth of periopathogens in vitro and maybe a potential risk to regeneration.

Keywords: periodontitis, antibacterial, barrier, membranes, regeneration, resorbable

INTRODUCTION

Regenerative periodontal therapy aims to predictably restore the tooth's supporting periodontal tissues (i.e. new periodontal ligament, new cementum with inserting periodontal ligament fibres and new bone) that have been lost due to periodontal disease or dental trauma. Nonsurgical and conventional surgical periodontal therapy may usually result in successful clinical outcomes such as probing depth reduction and gain of clinical attachment.

Guided tissue regeneration (GTR) is an attempt to regenerate lost periodontal structures through differential tissue response (AAP 1996). Placement of barriers to cover the bony defect and periodontal ligament excludes the gingival epithelium and gingival connective tissue from the root surface with the belief that they interfere with regeneration. The cells that repopulate the root surface after periodontal surgery will determine the type of attachment that forms during healing. It allows re-population of the defect by cells from the PDL (Fibroblasts, cementoblasts and osteoblasts). Barriers can also help to stabilize the clot, leading to enhanced regeneration.

It is the most common clinical procedure to facilitate the growth of new tissue and increase the bone volume. A wide range of barrier membranes (BM) are used for GTR and GBR procedures. Membrane exposure constitutes the major complication associated with GTR, with prevalence between 50% and 100%.[1] Once membrane exposure is clinically detected, efforts should be directed to prevent or treat local infection as many studies have shown that exposed membranes are contaminated by bacteria.[2,3] Contaminated membranes are associated with reduced clinical outcomes.

Thus post exposure the nature of the surface of the BM and the adsorption of organic materials from saliva or serum, could provide a potential interface for cell colonisation.[4] After placement, bacteria from the oral cavity may colonize the coronal part of the membrane. Frequently, this results in recession of the gingival tissues, which allows colonization of the membrane material further apically. In addition, "pocket" formation may occur on the outer surface of the membrane due to apical migration of the epithelium on the inner surface of the covering gingival tissue. This may allow bacteria from the oral cavity to colonize the subgingival area.

Certain bacteria have high adherence to collagen membranes with periodontal pathogens showing greater affinity.[5] Certain bacteria have high adherence to collagen membranes with periodontal pathogens showing greater affinity. The proteolytic activity of the bacteria may result in rapid degradation of the membranes and thus impair the regenerative potential.[6]

Antibacterial properties of amniotic fluid are well documented and the presence of many potentially antibacterial factors has been demonstrated.[7] No such factors have yet been found in amniotic membranes.

Talmi et al. (1991) have demonstrated an inhibitory effect of amniotic membranes on agar plates.[8] Kjaergaard et al. (2001) tested the antibacterial properties of amnion and chorion against diverse panel of bacteria. He concluded that amnion had marked inhibitory properties against most of the bacteria however on the other hand chorion showed a marginal inhibitory effect.[9] M. Parthasarathy (2014) confirmed the antimicrobial effect of both amniotic and chorionic membranes against several bacterial and fungal pathogens and found that among the two membranes, the maximum activity was recorded by amniotic membrane.[10] Slutzkey et al (2015) found that collagen barrier membranes possess no antibacterial properties and tested 3 different collagen BMs BioGuide (non cross-linked), OsseoGuard (cross-linked) and CopiOs (non cross-linked) and concluded that OsseoGuard (cross-linked) showed bacterial enhancing effect.[11]

The antibacterial activity if present can control inflammatory lesion and thus increase the life of the membrane and treatment

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How to cite this article: Prutha Vaidya, Swapna Mahale, Pallavi Badade, Alisha Panjwani, Agraja Patil, Ayushya Warang. Antibacterial efficacy of collagen and foetal barrier membranes. International Journal of Contemporary Medical Research 2016;3(8):2292-2294.

outcome.

The aim of this study is to investigate the antibacterial properties of collagen and foetal barrier membranes i.e amnion and chorion.

MATERIAL AND METHODS

The study was conducted in Maratha Mandal's NGH institute of dental sciences and research centre, Belgaum.

Membranes tested

- Collagen membrane (Healiguide)
- Chorion membrane (Tata memorial tissue bank)
- Amnion membrane (Tata memorial tissue bank)

Bacteria tested

- Staphylococcu *S aureus*
- Aggregatibacter actinomycetemcomitans
- Porphyromonas gingivalis

Method of bacterial growth evaluation

Direct Contact Test (DCT)

The DCT is measured by determining growth on a 96 well microtiter plate. The outgrowth kinetics in each well was recorded continuously by measuring optical densities at 650 nm at specific time intervals i.e 30 min, 60 min, 90 min, 120 min, 150 min, 180 min and finally at 24 hrs.

Two samples of each membrane were taken for each of the three organisms. This was designated as group A. They were attached to the walls of the wells. Bacterial suspension was placed on each piece of membrane. It was then incubated for 1 hour at 37 degree C for bacterial fluid suspension to evaporate and to ensure close and direct contact between membrane and the bacteria. After 1 hour the respective culture media for each strain was added to each of group A well and gently mixed. Then some amount of suspension was transferred from group A wells respectively to adjacent set of wells and these wells were designated as group B. The transfer was such that equal volume of liquid media was maintained in both experimental groups A and B so that the bacterial outgrowth could be monitored and compared, both in the presence and the absence of test membrane.

One set of three wells served as positive controls i.e they had similar bacterial inoculum of the three strains as in the duplicates of group A but without the membrane in the wells. It was processed similar to the experimental group.

One set of three wells formed the negative control and contained the test membranes as in experimental group A and an equal volume of uninoculated fresh media. This negative control was treated as baseline. Then the outgrowths were monitored at 650 nm at 30, 60, 90, 120, 150, 180 minutes and at 24 hrs. Data was recorded in optical densities.

Data processing

The OD values from the negative control well were considered baseline and were subtracted from the respective experimental data which were then plotted as growth curves. The curves for each well were analyzed, and a regression line was calculated on the ascending linear portion of the curve, expressed by the simple function $y = ax + b$. The formula of the linear portion provided two parameters: the slope, indicating growth rate; and the constant, correlating with the number of bacteria at time zero.

STATISTICAL ANALYSIS

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

RESULTS

Bacterial growth in presence of membranes

Table-1 indicates the growth rates derived from slopes of the linear regression. The growth of each bacteria on each membrane was compared to its respective positive control.

***S aureus* -** The membrane samples of collagen, amnion and chorion in the suspension did not disrupt the growth of *S aureus* as compared to control.

***P gingivalis* -** All three membranes did not disrupt the growth of P gingivalis as compared to control. However chorion membrane accelerated the growth of P gingivalis as compared to control group ($P < 0.05$).

***A actinomycetemcomitans* -** All three membranes did not disrupt the growth of *A actinomycetemcomitans* as compared to control. However chorion membrane accelerated the growth of *A actinomycetemcomitans* as compared to control group ($P < 0.05$).

DISCUSSION

The results showed that the membranes did not disrupt the growth of tested bacteria as compared to control groups. Thus they had no antibacterial properties. However chorion membrane accelerated bacterial growth. The DCT, which was used in this study, is an accepted method for testing antibacterial activities of dental materials. Most studies that tested the antibacterial properties of dental materials used the Agar Diffusion Test. An antibacterial activity measured by this kind of technique is not necessarily positive because insoluble components will show negative results.

Collagen is an important constituent of all the 3 membranes. Some of the bacteria that colonize the membranes were found to have the ability to rapidly degrade collagen by proteolytic activity.[6] In addition, the colonizing bacteria can pass through membranes accompanied by fibroblasts and giant cells or

	Positive Control	Collagen		*P* value	Chorion		*P* value	Amnion		*P* value
		Sample 1	Sample 2		Sample 1	Sample 2		Sample 1	Sample 2	
S. aureus	0.035	0.032	0.037	0.865	0.017	0.012	0.123	0.031	0.029	0.227
P. gingivalis	0.063	0.058	0.066	0.908	0.026	0.025	0.017 *	0.054	0.045	0.315
A. a	0.026	0.032	0.035	0.181	0.004	0.002	0.026*	0.029	0.035	0.457
* statistically significant (P value < 0.05)										

Table-1: Growth of bacteria

invade the membrane and colonize its internal surface.[12] Higher affinity of bacteria to certain materials is due to their increased hydrophilicity. It can be speculated that the collagen membrane used in this study was more hydrophilic due to its crosslinked structure.[11] Thus there was an enhanced growth of *S. aureus, P.gingivalis* and *A. actinomycetemcomitans* as compared to control, however it was not statistically significant.

Amniotic membranes have the ability to produce β-defensins, elafin and secretory leukocyte protease inhibitor. It also exhibits cystatin E, the analogue of cysteine proteinase inhibitor.[9] Thus in the present study amnion membrane reduced the growth of *P.gingivalis* whose one of the virulence factors is Gingipains which is a cysteine protease. However the dehydration process for clinical use decreases the concentration of antimicrobial factors and hence the reduction was not statistically significant. There is a lack of literature regarding the exact antimicrobial properties of chorion membrane. However a major constituent of chorion membrane is Laminin-5 which is a component of Extra Cellular Matrix with a high affinity for cellular adhesion. It has been hypothesized that there is an increased interaction between periopathogens and these ECM proteins leading to their increased growth. These interactions fail in presence of blood however during membrane exposure the risk increases. Thus in this study there was an increased growth of all 3 microorganisms, however there was a statistically significant increased growth of A.a and *P. gingivalis*.

CONCLUSION

In clinical situations where definitive closure of gingival flaps is not predicted or hard to achieve for example socket preservation procedures chorion membrane should be avoided. The collagen membrane tested had no antibacterial properties. Nonetheless, the crosslinked collagen membranes should be used with caution. Amnion membrane is a suitable membrane as it caused least bacterial growth and had some inhibitory effect on *P. gingivalis*. However, studies have showed that maintenance of good oral hygiene and minimal gingival inflammation demonstrated consistently better regenerative response. Hence the key to any successful regeneration is to provide an environment non conducive for the growth of harmful bacteria, and since their elimination is an impossible concept we can atleast reduce their load and attempt to prevent their excessive growth.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 23-06-2016; **Published online**: 25-07-2016