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Comparative evaluation of GTR membranes to assess their bacterial activity in vitro

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ABSTRACT

Background

Periodontal regeneration is a reproduction or construction of a lost or injured part in such a way that the architecture and function of the lost or injured tissues are completely restored. Guided tissue regeneration has been used to regain lost periodontal attachment around teeth. Microorganism can adhere to and colonize exposed membranes leading to the development of nidus of infection. Aim of this study is to evaluate adherence of bacteria Porphyromonas gingivalis over Amnion, Chorion and Collagen GTR membrane.

Methodology

The membranes were placed in tubes containing broth cultures and identical concentrations (1×10 8) of these bacteria at 37°C. At 4, 6,12 and 24 hrs. The bacterial cultures were decanted and the membranes in the tubes were agitated gently, in reduced transfer fluid for 4 times to remove non adherent bacteria. Each tube was sonicated for 30 sec in 10 ml RTF to detach adherent bacteria. The detached adherent bacteria were counted using a Petroff-Hausser chamber.

Result

P. gingivalis can adhere to Chorion, Amnion and Collagen membranes at interval of 2hrs, 4 hrs and 24 hrs.

Conclusion

P.gingivalis has higher affinity of adherence towards Chorion as compared to Amnion and Collagen membranes.

Keywords: GTR membrane, P. Gingivalis, Bacterial Contamination, Periodontal Regeneration.

INTRODUCTION

Melcher hypothesis stated that certain cell populations residing in the periodontium have the potential to make new periodontal ligament, cementum and alveolar bone [1]. Karring et al established Melcher hypothesis and verified it histologically. Result of their study suggests that when gingival epithelial cells or fibroblasts are excluded and periodontal ligament cells are allowed to migrate and populate in to the wound space, selected cell populations residing in the periodontium can produce new cementum, alveolar bone and periodontal ligament. The necessity for exclusion of epithelium and connective tissue cells

of the gingiva from wound led to development of periodontal devices know as barriers or membranes for guided tissue regeneration [2].

The first GTR membrane used in the periodontal surgery was cellulose acetate laboratory filter paper by Nyman et al in 1982 [3]. A variety of absorbable and non-absorbable biomaterials are being used clinically or are being tested experimentally for use with GTR procedures [4]. Although attempts were made to limit the exposure of these materials to the oral environment, because of flap dehiscence or gingival recession, exposure of the GTR membrane during the healing period is a common occurrence.

Microorganism can adhere to and colonize exposed membranes leading to the development of nidus of infection. Microbial adherence has been associated with infection and subsequent rejection of many biomaterials [5]. Pioneering bacteria which initially adhere to biomaterials can multiply and cause infection or provide a base for co-aggregation of secondary microorganisms leading to infection [6]. Furthermore, Selvig et al. reported that the extent of oral exposure and bacterial contamination of the expanded polytetrafluoroethylene (ePTFE) membrane at the time of removal may be an indicator of the long-term success or failure of the regenerative procedure [7].

Bacterial adhesion and colonization of exposed GTR membranes used in conjunction with implants, may cause the development of infection in the underlying treated tissues and result in partial regeneration or implant failure [8]. Selvig et al. (1992) and Machtei et al. (1994) suggested that the amount of bacteria found on regeneration membranes may be used as a predictor of the outcome of the regenerative treatment. They suggested that in order to ensure successful regeneration, periodontal pathogens should be controlled in the site of membrane insertion. Gingipains of *P. gingivalis* are responsible for the majority of the proteolysis including the collagenolytic activity of this bacteria [9]. GTR Membranes (Amnion, Chorion and Healiguide) used in this study were easily available, cost effective and biocompatible. To date, little research has been conducted on the comparison on GTR membranes to assess bacterial activity, hence this study was undertaken.

METHODOLOGY

Membrane selection

Three different sterile GTR materials were selected for this study.

1) Group 1: Chorion
2) Group 2: Amnion
3) Group 3: Healiguide (Collagen)

Microorganism selection and preparation

Oral microorganism was included in this study

P. gingivalis

The organisms were clinical isolates from Periodontitis patients preserved in frozen vials stored in liquid nitrogen at - 135°C. The organisms were defrosted, re-vitalized, and incubate the membranes (3 groups) in thioglycollate broth (Pg) for 2 hrs, 4 hrs and 24 hrs.

Experimental design

Membranes were cut into 6x8 mm rectangular form in a laminar flow hood. Both surfaces of the membrane specimens were vulnerable to bacterial exposure during immersion in a bacterial suspension. Four tubes per test membrane each containing 4.5 ml of appropriate broth medium and three pieces of the same experimental specimens were inoculated with 0.5 ml bacterial suspension at a concentration of 109 cells/ml. The final concentration of the organisms at 0 hour was 108 cells/ml. Identical test tubes containing the same membrane and broth media without bacteria were used as control. All the test tubes were incubated at 37°C in the appropriate gaseous environments.

After 4, 6, 12, and 24 hours, the media of three test tubes containing each kind of material were decanted into sterile bottles and the concentration of bacteria was counted with a Petroff-Hausser chamber. Ten milliliters (ml) of sterile RTF were added to each test tube and agitated gently for 20 seconds. The RTF was aspirated with a sterile dropper. Thereafter, 10 ml of fresh, sterile RTF was added into each test tube. The same procedure was repeated 4 times to remove non-adherent bacteria. Test tubes with Gram positive bacteria were sonicated at 10 kHz for up to 30 seconds in 10 ml RTF to dislodge adherent organisms from the membrane materials. The membranes with Gram negative bacteria were sonicated for 5 to 10 seconds to avoid possible lysis. The sonication

effluent with bacteria was counted using a Petroff-Hausser chamber under a phase-contrast microscope. Data were calculated in term of 107 cells/cm for each membrane. After each time interval, transfer the membranes to RTF (reduced transport fluid, 100µl) and agitate for 30secs. Identical uninoculated bottles were used as controls. Media of the inoculated bottles were changed every 24 to 48 hours depending on the growth of each microbial species as determined by turbidity. Each time the media were changed, the purity of the cultures was verified using phase-contrast microscope and standard cultural tests.Count the cells using Neubar counting chamber.

RESULT

During the experimental period, there was no evidence indicating any bacterial or fungal contamination of the well chambers. The effect of the barrier membranes on periodontal ligament fibroblast cell proliferation was counted by Naubar's chamber after 2, 4 and 24hours *in vitro*. The rate of bacterial proliferation with time was different among the membranes examined. Descriptive statistics such mean and SD was used. P-values in comparison with controls were determined by Post Hoc Dunnett Multiple Comparisons Test after ANOVA. A p-value less than 0.05 were considered as significant.

Group 1 Amnion showed adherence rate 2.87 ± 1.66, 3.95 ± 1.53 and 4.4 ± 1.07 after 2hours, 4hours and 24 hours respectively.

Group 2 Chorion showed 3.22 ± 1.39, 3.85 ± 1.39 and 3.22 ± 0.86 adherence rates after 2hours, 4hours and 24 hours respectively.

Group 3 Healiguide showed adherence rate 3.05 ± 1.78, 4.45 ± 1.46 and 4.46 ± 1.34 after 2hours, 4hours and 24 hours respectively.

P value after 2hour is 0.18, after 4hours is 0.07 and after 24 hours is 0.4 which means results of this study is not significant. *P.gingivalis* have ability to adhere all of three membranes but its attachment was greater with group 3 i.e. Healiguide when compared to the other two groups. Group 1 showed less adherence rate compared to group 2 and 3.

DISCUSSION

Clinically, attempts have been made to prevent exposure of barrier materials to the oral environment. However, due to a number of problems including unfavourable dental morphology, flap dehiscence, gingival recession, or incomplete flap closure, membrane exposure is a common sequel during the early healing phase. Exposure may permit a communication between the oral environment and newly forming tissues which may increase the potential for infection of the barrier. The adhesion of bacteria to mammalian tissue surfaces and biomaterials has been recognized as an important initial step in the pathogenesis of infection. Qualitative observation from many study demonstrated that microbes could colonize GTR membrane [10].

P.gingivalis is an anaerobic Gram-negative bacteria involved in the onset of inflammation and tissue destruction during periodontal disease. It can be found in small numbers in the oral cavity of healthy individuals. Pathology occurs when *P.gingivalis* binds to and accumulates on the tooth surface, leading to the development of a mixed biofilm, the expansion of the bacteria into the gingival sulcus and the formation of a periodontal pocket [11]. Since early degradation of collagen membranes is detrimental to the success of regenerative procedures, the inhibitory effect of chemical processes and materials on bacterial adherence and early degradation were examined. Recent studies that have examined modified resorbable GBR–GTR membranes with different antibacterial properties, suggested that membranes loaded with such materials might enhance GTR efficacy [12].

Studying oral bacterial adherence on tooth surfaces, Brecx et al found that the majority of the increase in the microbial mass comes from bacteria already present on the tooth surface and they in turn provided the basis of co-aggregation by secondary bacteria species. Early bacterial adhesion seems to be more important than secondary accumulation [13]. Therefore; this study only examined early bacterial adhesion. Passariello et al reported bacterial colonization in all 16 patients who underwent periodontal regeneration procedures by implantation of ePTFE [14]. Furthermore Selvig et al using SEM, observed bacterial contamination of ePTFE membranes [15].

Direct microscopic count can be done by Petroff-Hausser chamber, hemocytometer, electronic counters (such as Coutler counter), and radio-labeled bacteria that are counted by scintillation. In this study, we used the Petroff-Hausser chamber to count the adherent number of bacteria. This method is fast and easy to perform; however, some drawbacks are present since it may count both living and dead bacteria. Artefacts may also be counted along with the cells under microscopic examination.

Data from present study demonstrated that *P.gingivalis* have ability to adhere all three membranes after 2hours, 4hours and 24 hours. Results of this study stated that after 2 hours group 2 showed more adhesion than other groups. Group 3 showed least adhesion after 2 hours. Changes occurred after 4 hours; group 1 showed more adhesion than other membranes and group 2 showed least adherence. After 24 hours group 3 showed more adhesion and group 1 less than other.

CONCLUSION

The present study was done with the aim to compare three resorbable membranes (Amnion, Chorion and Healiguide) for evaluation of their fibroblastic activity, bacterial activity and resorbability. The three GTR membranes used in the present study differed in their structure and physical characteristics. Larger membrane porosity could allow for cell ingrowths within the membrane, thus enabling better tissue integration and cell growth within the membrane, but it also could result in reduced barrier function. In the present study *P.gingivalis* adhered to all three GTR membranes. The adherence increases as the exposure time of the membrane to the bacteria increases. Results obtained from the present *in-vitro* study cannot be directly extended to clinical situation; however, they do provide reproducible and reliable means for comparing and testing the antimicrobial activity of various membranes.

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