Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.50919-50922, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

EVALUATION OF ADHERENCE OF ORAL MICROORGANISMS TO TITANIUM MESH : AN INVITRO STUDY

***Krishnan Swathi, Savita, A. M., Naik Archana, R., Varghese Ammu and Prerana, G. K.**

Rajiv Gandhi University of Health Sciences, India

ARTICLE INFO

Article History:
Received 09th February, 2017
Received in revised form
11th March, 2017
Accepted 06th April, 2017
Published online 31st May, 2017

Key words:

Titanium mesh,
Porphyromonasgingivalis,
Guided bone regeneration,
Bacterial adherence.

ABSTRACT

Background: The ultimate goal of any periodontal therapy is the regeneration of lost periodontal tissues. Guided bone regeneration (GBR) is a regenerative procedure that uses barrier membranes to direct the growth of new bone. The superior mechanical properties of Titanium-mesh make it optimal for successful GBR. However many problems like early exposure and resultant microbial on growth on its surfaces leads to the failure of regenerative therapy.

Aim: The aim of this study is to evaluate and compare the bacterial adherence of four strongly adhesive periopathogenic bacteria (Actinomyces viscosus, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Streptococcus mutans) to Titanium mesh and Type 1collagen membrane.

Methods and Materials: Four tubes with each containing the test membrane and 3 ml of appropriate broth medium (inoculated with 0.5 ml bacterial suspension) were incubated for different time inervals (6, 12, 24, 48, 72 hours) and the bacterial adherence on the membrane was counted.

Statistical analysis: The results were averaged (mean ± standard deviation) for each continuous parameter. Analysis of variance (ANOVA) and unpaired t test analysis were used.

Results: The difference between all the bacterium for Titanium mesh and collagen membrane were statistically significant at 48 hours. Porphyromonas gingivalis showed statistically significant bacterial adherence difference at both 48 and 72hours and Actinomyces viscous, at 24 hours to Ti mesh.

Conclusions: Oral microorganisms vary in their ability to adhere to biomaterial membranes. In comparison to collagen membrane, Ti mesh has significantly lower Actinomyces viscosus adherence at 24 hours and lower Porphyromonas gingivalis adherence at 48 and 72 hours.

Citation: Krishnan Swathi, Savita, A. M., Naik Archana, R., Varghese Ammu and Prerana, G. K. 2017. "Evaluation of adherence of oral microorganisms to titanium mesh : an Invitro study", *International Journal of Current Research*, 9, (05), 50919-50922.

INTRODUCTION

Periodontitis is a multifactorial inflammatory disease resulting in the loss of tooth supporting structures. The ultimate goal of any periodontal therapy is the regeneration of lost periodontal tissues. Periodontal regeneration is the formation of new cementum, periodontal ligament and alveolar bone following periodontal surgery. [1] The most important factor that determines the long term prognosis of any periodontal regenerative techniques is adequate bone volume.[2] Various methods have been developed inorder to augment the bone volume; however most of these methods such as distraction osteogenesismay often leave undesirable tissue effects.[2] The use of osteoinductive as well as osteoconductive bone grafting approaches have provided most successful results in bone regeneration, but thefailure in stabilizing the bone grafts at the defect site and the risk of soft tissue in growth onto the defect space may reduce the predictability of bone augmentation.[3,4]

****Corresponding author: Krishnan Swathi***
Rajiv Gandhi University of Health Sciences, India.

Guided bone regeneration (GBR) is a regenerative procedure that uses barrier membranes to direct the growth of new bone at sites with insufficient volumes or dimensions of bone. The basic principle of GBR involves the placement of mechanical barriers to protect blood clot and to isolate the bone defect from the surrounding connective tissue, thus providing bone forming cells with access to a secluded space intended for bone regeneration.[5,6] GBR membranesalong with bone grafts improves the predictability of bone augmentation and provides long-term stability to the newly augmented bone. GBR membrane should possess ideal characteristics such as biocompatibility, occlusivity, spaciousness, clinical manageability and the appropriate integration with the surrounding tissue which helps to provide the maximum membrane function and mechanical support to the tissue during bone formation. [7] A membrane for bone generation should be selected based on a thorough understanding of the benefits and limitations inherent to the materials in relation its functional requirements in the specific clinical application. The superior mechanical properties of Titanium(Ti)-mesh make it optimal for successful GBR. Its rigidity provides extensive

space maintenance and prevents contour collapse; its elasticity prevents mucosal compression; its stability prevents graft displacement and its plasticity permits bending, contouring and adaptation to any unique bony defects.[8,9] However, many problems still remain and need to be resolved to increase the predictable nature of Ti mesh. Most problems with Ti mesh arise from their exposure and from microbial on growth on the surfaces. Microorganisms can adhere and colonize on an exposed Ti mesh thus developing a nidus of infection and thusleads to the failure of regenerative therapy. Even though various studies are reported, the extent of oral exposure and bacterial contamination of both absorbable and non-absorbable biomaterials, there is limited literature available regarding the bacterial contamination on Ti mesh.[10]The purpose of this study was to evaluate and compare the bacterial adherence of four strongly adhesive periopathogenic bacteria (*Actinomycesviscosus, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Streptococcus mutans,*) to Ti mesh[#]and Type 1collagen membrane[*].

MATERIALS AND METHODS

Two different GBR membranes were selected for the study: Timesh and Type 1 collagen membrane. Ti mesh, non-resorbable and bio-inert membrane is assigned as the test group membrane and Type 1 collagen membrane, resorbable membrane assigned as positive control group.

Bacterial preparation

The log phase broth cultures of selected bacterium such as *S. mutans, A. viscosus, A. actinomycetemcomitans* and *P. gingivalis* have been harvested by centrifugation at 3500 rpm for 20 minutes and resuspended in reduced transport fluid (RTF) and dispersed by a Vortex mixer for 30 seconds.

Study design

Type 1 collagen membrane is assigned as group A (control group) and Ti mesh membrane assigned as group B (test group). Each Membrane is cut into16 rectangular pieces (Dimensions : 4x4mm; 4 pieces each for each bacteria) and prepared in a laminar flow hood (Figure 1).

Each tube are taken with 3 ml of appropriate broth medium and 0.5 ml bacterial suspension; inoculated at a concentration of 10^9 cells/ml. The experimental membranes are kept immersed in the bacterial suspension and thus bacterial growth can be detected on both of its surfaces. The final concentration of the organisms at 0 hour was10^8 cells/ml (Figure 2 and 3). All the test tubes were incubated at 37°C. After 4, 6, 12, 24, 48, 72 hours, the media of test tubes containing each bacterial specimen were decanted into sterile bottles and the concentration of bacteria was counted with a Petroff-Hausser chamber. Prior to counting, RTF is added to each test tube, vibrated gently for 30 seconds and aspirated with a sterile dropper. The same procedure is repeated 4 times to remove non-adherent bacteria. Test tubes with Gram positive bacteria are sonicated at 10 kHz for up to 30 seconds in 15 ml RTF to dislodge adherent organisms from the membrane materials. The membranes with Gram negative bacteria are sonicated for 5 to 10 seconds to avoid possible lysis. The sonication effluent with bacteria is counted using a Petroff- Hausser chamber using quantitative phase-contrast microscopy. Data were calculated in terms of 10^7 cells/cm^2 for each membrane.

Statistical analysis

The results were averaged (mean ± standard deviation) for each continuous parameter. Analysis of variance (ANOVA) was used to test differences of the mean bacteria adherence among the types of the bacteria with each membrane. Unpaired t test analysis was used to test differences of the mean bacteria adherence among the membranes at each type of bacteria. Differences were considered significant when the ANOVA value of Bonferroni adjusted t test was less than 0.05.

RESULTS

A total of 16 samples of each membrane were used for the study. The adherence of bacteria was tested in 6, 12,24,48,72 hours' time interval. Type 1 collagen membrane is assigned as group A (control group) and Ti mesh membrane represented as group B(test group).

Table 1. Intragroup comparison of bacterial adherence to Titanium mesh and collagen membrane

Time		A.actinomycetemcomitans	S.mutans	P,gingivalis	A.viscosus	F value	P Value
24 hours	Ti mesh		1.22± 0.22		1.3±0.055	164.9	
	Collagen membrane	0.91±0.036	1.64± 1.408	0.13±0.082	1.85± 0.3	4.683	0.022*
48 hours	Ti mesh	1.76±0.656	2.44±0.63	0.63±0.0211	3.86±1.85	6.862	.006*
	Collagen membrane	1.73± 0.25	2.75±0.42	0.96±0.19	3.72±0.74	28.13	0.01*
72 hours	Ti mesh	2.2±1.08	3.02±1.56	1.68±0.16	4.28±1.09	4.269	0.029*
	Collagen membrane	3.41±0.93	3.88±1.08	2.8±0.461	3.96±0.43	1.87	0.18

The bacterial adherence counts are represented as means ± SD= 10^7 cells/mm^2 ; *p value<0.05 (statistically significant)

Table 2. Intergroup comparison of bacterial adherence to Titanium mesh and collagen membrane

Time		A.actinomycetemcomitans	S.mutans	P,gingivalis	A.viscosus
	Ti mesh	-	1.22± 0.22	-	1.3±0.055
24 hours	Collagen membrane	0.91±0.036	1.64± 1.408	0.13±0.082	1.85± 0.3
T value			0.5806		3.606
P value			0.5827		0.0113*
	Ti mesh	1.76±0.656	2.44±0.63	0.63±0.0211	3.86±1.85
48 hours	Collagen membrane	1.73± 0.25	2.75±0.42	0.96±0.19	3.72±0.74
T value		0.086	0.818	3.452	0.1405
P value		0.9341	0.44	0.013*	0.892
	Ti mesh	2.2±1.08	3.02±1.56	1.68±0.16	4.28±1.09
72 hours	Collagen membrane	3.41±0.93	3.88±1.08	2.8±0.461	3.96±0.43
T value		1.698	0.90	2.772	0.546
P value		0.1404	0.399	0.0323*	0.604

*p value< 0.05 (statistically significant)

Figure 1. Collagen membrane and Ti mesh (16 pieces- 4x4mm) prepared in laminar flow hood

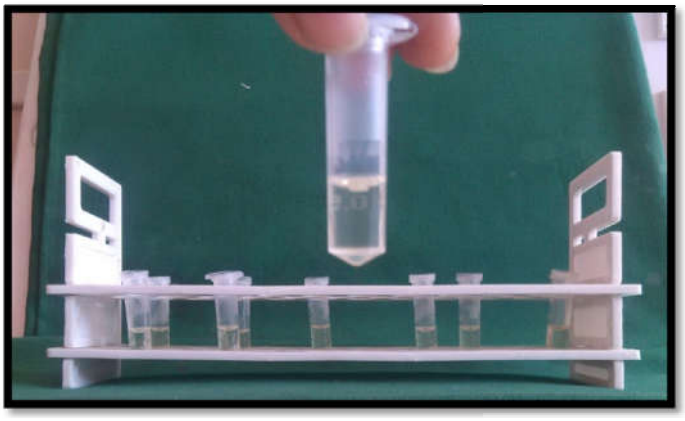

Figure 2. Analysis of microbial adherence on collagen membrane

Figure 3. Analysis of microbial adherence on Ti-mesh

Bacterial adherence to membranes

The mean adherence values of group A and B at 48hours for *A.actinomycetemcomitans* were 1.73± 0.25 and 1.76±0.656 respectively and at 72hours is 3.41±0.93 and 2.2±1.08 respectively. The mean adherence values of group A and B at 24hours for *S.mutans* were 1.64± 1.408and 1.22± 0.22respectively; at 48hours is 2.75±0.42and 2.44±0.63 respectively and at 72 hours is 3.88±1.08 and 3.02±1.56 respectively (Table 1 and 2). The mean adherence values of group A and B at 48hours for *P.gingivalis* were 0.96±0.19 and 0.63±0.0211respectively and at 72hours is 2.8±0.461 and 1.68±0.16 respectively. The mean adherence values of group A and B at 24hours for *A.viscosus* were 1.85± 0.3and 1.3±0.055 respectively; at 48hours is 3.72±0.74and 3.86±1.85 respectively and at 72 hours is 3.96±0.43and 4.28±1.09respectively (Table 1 and 2). Normality of the adherence scores were examined by using Shapiro-wilk test. The difference between all the bacterium for Ti mesh and collagen membrane were statistically significant at 48 hours. However for Ti mesh, the difference was statistically significant at 72hours and for collagen membrane the difference was statistically significant at 24 hours (Table 1).

Intermembrane analysis

The intermembrane analysis was done for each bacterium. In case of *P.gingivalis*, it was found that there is statistically significant bacterial adherence difference at both 48 and 72hours (Table:2). In case of *A.viscous,* bacterial adherence difference was found statistically significant at 24 hours and not significant at 48 and 72 hours (Table:2). However for both *A.actinomycetemcomitans* and *S.mutans,* it was not statistically significant at 24, 48 and 72 hours (Table 2).

DISCUSSION

The concept of GBR using non resorbable membrane that acts as a barrier to prevent soft tissue invasion into the defect and forms an enclosed space for guiding the bone regeneration process is one of the common bone reconstruction method in dentistry. One of the disadvantages while using most of the barrier membranes is collapse of the membrane towards the bone defect reducing the space needed for bone regeneration.[10] This problem can be overcome with the use of more stiffer membranes like Ti mesh. Titanium has extensive applications due to its high strength, density, low weight and resistance to corrosion and high temperatures.[10]In 1969, Boyne et al introduced titanium in the form of Ti mesh and implicated its role in bone regenerative procedures.[11] However, as the mesh has to be placed for an extensive period of time it is more susceptible to exposure as well as in soft tissue growth; both leading to the failure of regenerative procedure. The titanium has been proved to be a membrane with biocompatibility and resistance to various microbial infections. However once the mesh is exposed it might act as a nidus for the microbial adhesion leading to plaque formation and ultimately to the failure of regeneration. The initial step in the progression of pathogenesis of periodontitis is mainly the bacterial adhesion on the biomaterials used in the regenerative procedures.[11] 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 bacterial species. Early bacterial adhesion seems to be more important than secondary accumulation.[12] Therefore, this study only examined early bacterial adhesion. In the study, specifically adhering periodontal pathogens like *A.viscosus, A.actinomycetemcomitans, P.gingivalis, S.mutans* are selected.

The difference between all the bacterium for Ti mesh and collagen membrane were statistically significant at 48 hours. However for Timesh, the difference was statistically significant at 72hours and for collagen membrane the

difference was statistically significant at 24 hours. Among the gram positive organisms, both *S.mutans* and *A.viscosus* showed adherence at 24hours onwards and there were significant difference between titanium mesh and collagen membrane at 24 hours. No significant difference found at any other time intervals. This showed that eventhough it took time for *A.viscosus*to adhere to Ti mesh, once the adherence initiated,the organism multiplied exponentially and reached almost similar level with that on collagen membrane. *S.mutans* showed higher initial adherence to Ti mesh which is almost similar to that on collagen membrane. This is in contrast with earlier studies [11] where *S.mutans* showed stronger adhesion at 12 and 24 hours than *A.viscosus.*

P.gingivalis has showed adherence to both membranes from 48 hours onwards and there were statistically significant differenceat 48 and 72 hours' time intervals. This showed that *P.gingivalis* has lower adherence to Ti mesh when compared to collagen membrane and the progression at further time interval is also slower. In case of *A.actinomycetemcomitans,* the adherence to both membranes was almost similar and initiated from 48 hours onwards. Our results are in favor with other studies where they found *P.gingivalis* has strongest collagenolytic activity when compared other oral bacteria.[11,13] It also has to be noted that most of the periodontal pathogens are associated with infective endocarditis. Since GBR is a long term treatment, the risk of bacterial adherence in infective endocarditis patients can be considered as an important factor in determining and selecting the appropriate membrane.

The limitation of this study is mainly that we performed direct microscopic count of the individual microbial cells on the membranes. We used Petroff-Hausser chamber to count the number of adhere bacteria which is less technique sensitive and but nonspecific as it counts both living and dead bacteria and artifacts may also be counted along with the cells under microscopic examination.[11]

This study has been done only for four specifically selected periodontal bacteria and also the involvement of all other microbial pathogens is not evaluated. As oral cavity hosts various complex organisms, the adherence of each organism will be influenced by many other microbes. Therefore further future vivo studies are indicated to analyze oral bacterial adherence to Ti mesh and the effectiveness of antibiotic coated titanium mesh in bone regenerative procedures.

Conclusion

Within all the limitations, the following conclusions can be drawn: 1) Oral microorganisms, such as *S.mutans, P.gingivalis, A.actinomycetemcomitans* and *A.viscosus* can adhere to both Ti mesh and collagen membrane within 48hours under favourable conditions and adherence is more faster in collagen membrane in comparison to Ti mesh. 2) Ti mesh have significantly ($P < 0.05$) lower *A.viscosus* adherence than collagen membranes at 24 hours. 3) Collagen membranes have significantly higher *P gingivalis* attachment at 48 and 72 hours when compared to the other two membranes. Future studies are required to evaluate the clinical applicability of the study in periodontal regenerative procedures.

REFERENCES

1. Wang HL, Cooke J. 2005. Periodontal regeneration techniques for treatment of periodontal diseases. *DentClin North Am*., 49:637-59.
2. Ilizarov GA. 1989. The tension-stress effect on the genesis and growth of tissues:part I. The influence of stability of fixation and soft tissue preservation. *ClinOrthopRelat Res*., 238:249-81.
3. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. 2012. Stem cella in dentiatry-part I:stem cell sources. *JProsthodont Res*., 56:151-65.
4. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. 2012. Stem cella in dentiatry-part II:stem cell sources. *JProsthodont Res*., 56:151-65.
5. Burchardt H. 1983. The biology of bone graft repair. *ClinOrthopRelat Res*., 174:28-42.
6. Hammerle CHF, Karring T. 2000. Guided bone regeneration at oral implant sites. *Periodontol*., 17:151-75.
7. Scantlebury TV. 1982-1992: a decade of technology development for guided tissue regeneration. *J Periodontol*., 64:1129-37.
8. De Santana RB, de MattosCM, FrancischoneCE, Van Dyke T. 2010. Superficial topography and porosity of an absorbable barrier membraneimpacts soft tissue response in guided bone regeneration. *J Periodontol*., 81:926-33.
9. Degidi M, Scarano A, Piattelli A. 2003. Regeneration of the alveolar crest using titanium micromesh with autologous bone and a resorbable membrane. *J Oral Implantol*., 29:86.
10. Rakhmatia YD, Ayukawa Y, Furuhashi A, Koyano K. 2013. Current barrier membranes: Titanium mesh and other membranes for guided bone regeneration in dental applications. *JProsthodontol Res*., 57:3-14.
11. Wang HL, Yuan K, Burgett F, Shyr Y, Syed S. 1994. Adherence of Oral Microorganisms to Guided Tissue Membranes: An In Vitro Study *J Periodontol*., 65:211-8.
12. Brecx M, Theilade J, Attstrom R. 1983. Anultrastructural quantitative study of the significance of microbial multiplication during early plaque growth. *J Periodont Res*., 18:177-86.
13. Mayrand D, Grenier D. 1985. Detection of collagenase activity in oral bacteria. *Can J Microbiol*., 31:134-8.

****