"COMPARISION OF THE EFFICACY OF AMNIOTIC MEMBRANE V/S BUCCAL FAT PAD IN TREATMENT OF ORAL SUBMUCOUS FIBROSIS"

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By

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I hereby declare that this dissertation entitled **"COMPARISION OF THE EFFICACY OF AMNIOTIC MEM BRANE V/S BUCCAL FAT PAD IN TREATMENT OF ORAL SUBMUCOUS FIBROSIS"** is a bonafide, & genuine research work carried out by me under the guidance of **DR. HEMANT MEHRA, Reader** and **DR. HEMANT GUPTA, Professor & Head** Department of Oral& Maxillofacial Surgery, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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LIST OF ABBREVIATIONS

OSMF	Oral submucous fibrosis
AM	Amniotic membrane
KGF	Keratinocyte growth factor
TGF	Transforming growth factor
NGF	Nerve growth factor
EDGF	Epidermal derived growth factor
BFP	Buccal fat pad
RT PCR	Real time polymerase chain reaction
TIMPs	Tissue inhibitor of metalloproteinase
IL	Interleukin
IFN	Interferon
NO	Nitric oxide
NFκB	Nuclear factor kappa B
FKHR	Fork head in rhabdomyosarcoma
HLM	Human limbal myofibroblasts
RUNX2	Runt related transnscription factor 2
AP	Alkaline phosphatase
BMP	Bone morphogenetic protein
mRNA	Messenger ribonucleic acid
HAM	Human amniotic membrane
hAECs	Human amnion epithelial cells
hAMSC	Human amnion mesenchymal stromal cells
5-FU	5-fluorouracil

AMSC	Amniotic membrane-derived mesenchymal stem cell
CM	Conditioned medium
AMTCs	Amniotic mesenchymal tissue cell
tMFG	Temporalis muscle-fascia graft
TMJ	Temporomandibular joint
SMA	Smooth muscle actin
CD	Cell Diffrentiation
GCSF	Granulocyte-colony-stimulating factor
AMG	Amniotic membrane graft
TT-FDAM	Trehalosetreated freeze-dried amniotic membrane
CSDC	Corneal stroma derived cells
HbSAg	Hepatitis B antigen
HIV	Human immunodeficiency virus
HGF	Human growth factor
GTR	Guided tissue regeneration
VAS	Visual analog scale
HVAS	Horizontal visual analog scale
VVAS	Vertical visual analog scale
SPSS	Statistical Package for Social Sciences
Acc	According
v/s	Versus
Mm	Milli meter
BT	Bleeding time
CT	Clotting time

Hb	Haemoglobin
TLC	Total leucocytes count
DLC	Differential leucocyte count
ESR	Erythrocyte sedimentation rate
PP	Post prandial
MMO	Maximum mouth opening

ABSTRACT

Oral & maxillofacial surgery deals with wide range of oral defects, wound closure, tissue resection, and tissue reconstruction which involves tissues of dynamic zone, area of esthetic concern where ablative surgery have been done. The purpose of our study is to use amniotic membrane for closure of post surgery defect in patient of Oral submucous fibrosis to utilize its growth factor and scaffold nature for effective healing and to evaluate effectiveness of amniotic membrane in treatment outcome. Objectives are to compare post-operative mouth opening, healing of AM & BFP. To evaluate surgical reliability of the procedure and any complications occurring in both the groups, if any.

Diagnosed patients with OSMF are divided into two surgical site (n=5patients) Group I - Left side buccal mucosa in which resection of fibrous band with coronoidectomy followed by reconstruction of the mucosal defect with BFP. Group II - Right side buccal mucosa in which resection of fibrous band with coronoidectomy followed by reconstruction of the mucosal defect with Freeze dried irradiated Amniotic Membrane. Preoperative assessment included a thorough history and physical examination, measurement of maximal incisor opening (MIO), presence or absence of 3rd molars, extent & severity of fibrosis and causative factors / deleterious habits.

This study suggested that in comparison to buccal fat pad flap, the HAM graft is a better option for oral reconstruction in terms of infection, graft failure, MMO, inflammation, pain and supplenness. Although the number of cases was small, outcome indicated that the HAM is biologically ideal graft for oral wounds and could be used as clinical alternative for various repair surgery for oral defects.

INTRODUCTION

OSMF is an insidious, chronic disease affecting any part of the oral cavity & sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with juxtaepithelial inflammatory reaction, followed by a fibroelastic change of the lamina propria with epithelial atrophy, leading to stiffness of the oral mucosa and causing trismus and inability to eat.[1]

Various treatment of Oral Submucous Fibrosis has been proposed in attempts to improve the oral opening by medical as well as surgical means, which might exacerbate the disease by increased scarring. A wide range of treatments, medical management, surgical management, and physiotherapy have been attempted in the past, with varying degrees of benefit. Various modalities are mentioned but each has its own limitations.[2]

Amnion allograft is been used in field of medicine for its exceptional wound modulating properties. In the field of dentistry there are only limited number of reports which proposed its properties in healing of oral wounds. It is a membrane that surrounds & protect an embryo by building amniotic sac.[3]

Human amniotic membrane (AM) is being used as a biomaterial for surgical reconstruction for nearly 100 years. In 1910, Davis AM was used to treat burns & yielded efficient results as a temporary biological wound dressing. In 1913 Stern and Sabella studied the accelerative effect of the membrane on epithelialization and the

reduction in pain when it was applied on burnt or ulcerated sites. With this application of AM to the oral mucosa, Samandari et al performed mandibular vestibuloplasty using fresh AM, and reported that the enhancement of wound healing was observed.[4,5]

Amnion has three major layer: A single epithelial layer, thick basement membrane, and an avascular mesenchyme. With improving in the processing & storage technologies, amniotic membrane has been in use in various fields of medicine including Reconstruction of oral cavity, bladder and vagina, arthroplasty, management of burns and so on. Because of its inherent wound modulating property amnion allograft may be used to enhanced wound healing and enable tissue regeneration.[3]

The amniotic membrane is reported to have anti-inflammatory, antimicrobial properties and contains many growth factors like keratinocyte growth factor (KGF), Basic fibroblast growth factor, transformin growth factor (TGF-), NGF & epidermal derived growth factor (EDGF), which promote healing by providing natural healing environment .[6]

Fresh amnion has been used generally but frozen, dried, irradiated and lyophilized preparations have also been tried. The mesenchymal face of the amniotic membrane is to be applied to the wound surface as suggested.[7]

Buccal pad of fat is an encapsulated, rounded, biconvex fatty tissue which is used as an appropriate interpositional graft in surgical management of OSMF. The rich blood supply and ease of harvestation makes it an ideal flap for reconstruction after resection of bands in OSMF. The anatomical position which is in the close proximity to the buccal defect, its rich constant blood supply, more resistant nature to infection with no donor site morbidity and no extra oral scarring has made the BFP the preference graft.

Oral & maxillofacial surgery deals with wide range of oral defects, wound closure, tissue resection, and tissue reconstruction which involves tissues of dynamic zone, area of esthetic concern where ablative surgery have been done. The purpose of our study is to use amniotic membrane for closure of post surgery defect in patient of Oral submucous fibrosis to utilize its growth factor and scaffold nature for effective healing and to evaluate effectiveness of amniotic membrane in treatment outcome.

Hence in the present study, two modalities, with BFP grafting and with amniotic membrane covering as a wound dressing material was compared for the coverage of the buccal defect which is created after surgical removal of the fibrotic bands.

AIM AND OBJECTIVES

AIM

The aim of this study is to compare the efficacy of freeze dried irradiated amniotic membrane versus BFP as grafting material in surgical management of Oral Submucous Fibrosis

OBJECTIVES

The objectives of the present study are-

- To compare the healing with amniotic membrane and BFP grafting in reconstruction of defects in OSMF.
- To compare post-operative (increase in mouth opening)
- To evaluate complications occurring in both the groups, if any.
- Assessment of surgical reliability of the procedure.

REVIEW OF LITERATURE

AMNIOTIC MEMBRANE

STRUCTURE & PROPERTIES

Talmi YP, Sigler L, Inge E, Finkelstein Y et al (1991)[8] tried to establish the possible existence of potential antibiotic factors in amnion. Complete growth inhibition of all organisms was observed immediately under the amniotic membrane discs. This results provide evidence for the hypothesis that the antimicrobial effect of amniotic membranes in vitro is due to their close adherence to the wound surface.

Hao Y, Ma DH, Hwang DG, Kim WS et al (2000)[9] this was done to identify & observe the potential antiangiogenic and anti-inflammatory proteins expressed in human amniotic membrane tissue. RT-PCR results concluded that both human amniotic epithelial and mesenchymal cells express interleukin-1 receptor antagonist, all four TIMPs, collagen XVIII, and interleukin-10. Thrombospondin-1 expressed in all of the epithelial cell specimens and in one out of five mesenchymal cell specimens. Immunohistochemistry studies on freshly prepared amniotic membrane confirmed that all members of the TIMP family were present in epithelial and mesenchymal cells as well as in the compact layer of the amniotic stroma. Finally concluded that human amniotic membrane epithelial and mesenchymal cells express various antiangiogenic and anti-inflammatory proteins.

Kim JS, Kim JC (2000)[10] illustrated the presence of proteinase inhibitors which may facilitate wound healing. Thrombospondin-1, an antiangiogenic factor, is secreted from the epithelium of amniotic membrane. IL-1α and IL-1β, two very potent

proinflammatory mediators, there activity were suppressed by the stromal matrix of the amniotic membrane.

Shimmura S, Shimazaki J, Ohashi Y (2001)[11] observed when applied as a patch in vivo, amniotic membrane entraps T lymphocytes.

Estellés A, Grancha S, Gilabert J, Thinnes T et al (2004)[12] observed that transplantation of amniotic membrane as a temporary or permanent graft, improve epithelial wound healing and exerts potent anti-inflammatory and anti-scarring effects on the ocular surface. They aimed to recreate such a fetal environment to exert these actions by encasing the surgical site from the host environment. Thus concluded that the amniotic membrane can be used an ideal substrate for engineering different types of ocular surface tissues for transplantation.

Li W, He H, Kawakita T, Espana EM et al (2006)[13] observed the mechanism whereby AM induces macrophage apoptosis in vitro. Study concluded that AM stromal matrix produce apoptosis of IFN-gamma on activated, but not non-activated macrophages, not via generation of NO, but by downregulation of anti-apoptotic NF-kappaB and Akt-FKHR signaling pathways.

Manuelpillai U, Moodley Y, Borlongan CV, Parolini O (2011)[14] studied that in addition to the placenta, umbilical cord and amniotic fluid, the amniotic membrane is emerging as an immensely valuable and easily accessible source of stem and progenitor cells. They evaluated the effect exerted by the amniotic membrane on tissue inflammation and fibrosis.

Tehrani FA , Ahmadiani A, Niknejad H (2013)[15] illustrated antibacterial properties of the AM after preservation. Antibacterial property of the fresh AM were compared with cryopreserved and freeze-dried AM by modified disk diffusion method. The cryopreservation and freeze-drying procedures markly decreased elafin which showed that antibacterial property is not limited to the effects of amniotic cells and the other components such as extracellular matrix may help in contribution in this effects. Study's result showed that the preserved AM is a proper substitute of the fresh AM to be used in clinical situations.

Lopez AD, Lucio VM, Lopez JS, Sanchez ER et al (2014)[16] observed that the use of AM significantly inhibited the synthesis and secretion of proinflammatory cytokines on human limbal myofibroblasts (HLM). This was suggestive that AM generates some kind of anti-inflammatory microenvironment and specific inhibition of NFκB nuclear translocation on infected corneal tissue would reduce the inflammation and explain the effectiveness of transplanting AM on herpetic stromal keratitis.

Lockington D, Agarwal P, Young D, Caslake M et al (2014)[17] investigated the free radical scavenging properties of AM. They showed that AM is able to remove reactive oxygen species from its environment. Proving antioxidant capacity in AM provides evidence for use as a free radical scavenger which requires more research.

Chen H, Lai DR, Lian SL, Lin LM. (2014)[18] showed the use of fresh or stored frozen amnion as a single layer over the area to be dressed. It was applied with a protective stent on the wound for 5-7 days to prevent dislodgment after excision of lesions. The most noticeable features were the immediate adherence, marked relief of pain and protection against superficial bacterial infections. Similarly, the recipient

sites also showed re-epithelialization with pinkish covering mucosa and with no significant scar formation after one month.

STEM CELL PROPERTIES

Lindenmair A Wolbank S, Stadler G, Meinl A et al (2010)[19] evaluated the differentiation potential of AM in Toto with its sessile stem cells. Under osteogenic conditions, AM-biopsies mineralized successfully and by day 28 the majority of cells expressed osteocalcin. This was confirmed by a significant rise in calcium contents, increased AP activity, and induction of RUNX2, AP, BMP-2 and BMP-4 mRNA expression. They study reached to the conclusion that stem cells within human AM can successfully be driven along the osteogenic pathways while residing within their natural environment.

Prado SD, Lopez EM, Gomez TH, Cicione C et al (2011)[20] studied that the human amniotic membrane (HAM) is a highly abundant and easily available tissue with low immunogenicity & with high anti-inflammatory properties and these cells can be isolated without the sacrifice of human embryos. Since it is discarded post-partum it can be brought in use for regenerative medicine and cell therapy. Amniotic membranes is used extensively as biological dressings graft in ophthalmic, abdominal and cosmetic surgery. HAM contains two cell types, from different embryological origins, which shows some characteristic properties of stem cells. Human amnion epithelial cells (hAECs) derived from the embryonic ectoderm, where as human amnion mesenchymal stromal cells (hAMSCs) were derived from the embryonic mesoderm. Both having similar immunophenotype and multipotential for in vitro differentiation into the major mesodermal lineages but differ in cell yield. Therefore,

HAM has been used successfully in cell therapy or regenerative medicine to treat damaged or diseased tissues.

ANTICANCER PROPERTIES

Seo JH, Kim YH, Kim JS (2008)[21] focused on AM properties like antiangiogenic activity, immunoregulatory activity, and pro-apoptotic activity and their applications in tumor biology. Angiogenesis seen in tumor growth and destruction of the immunologic barrier is an inevitable process in tumor invasion and metastasis. Tumor cell killing is seen due to activation of pro-apoptotic pathway. Therefore, if we could apply this characteristics of the amniotic membrane to tumor biology, we may encounter a breakthrough within the sector of cancer treatment. For example, if a tumor mass might be enclosed by the amniotic membrane surgically, the expansion of tumor could be retarded. Further experiments and clinical trials should be conducted to verify such hypotheses.

Niknejad H, Yazdanpanah G, Mirmasoumi M, Abolghasemi H_et al (2013)[22] observed that the innermost layer of the amniotic membrane (AM) is considered as a suitable medium for cancer therapy. It was observed that unknown substances in the AM induce apoptosis in cancer cells and inhibit angiogenesis in tumors. HSP90 inhibits apoptosis in cancer cells. Based on other effects of the amniotic membrane ingredients and HSP90, they hypothesized that possible mechanism of the AM anti-cancer effects via inhibition of HSP90.

Niknejad H Khoei M, Peirovi H, Abolghasemi H_ (2014)[23] study showed comparison between the anti-cancer effect of amniotic epithelial cells and the whole

AM. Study concluded that amniotic epithelial cells with anti-angiogenic properties and induction of apoptosis, can behave as cancer therapeutic agents in the near future.

Niknejad H, Yazdanpanah G(2014)[24] reviewed anti-cancer property of the amniotic membrane. Based on some of the evidences, it is hypothesized that induction of apoptosis in cancer cells is originated from amniotic epithelial cells and cancer cell cycle arrest due to amniotic mesenchymal cells. They hypothesized that apoptosis and cell cycle arrest in cancer cells was induced by amniotic membrane due to release of soluble factors from amniotic cells.

ANIMAL STUDIES

Guler R, Uran N, Dilek FH (1993)[25] evaluated the wound healing effect of lyophilized amniotic membrane, which act as biological material as compared with control group with uncovered wound. Full thickness wounds were created on the dorsal regions of rats. They observed both microscopically and histopathologically that the graft has an accelerating effect on epithelialization and that the membrane is biodegradable.

Maral T, Borman H, Arslan H, Demirhan B, Akinbingol G et al (1999)[26] studied on the comparison on effectiveness of Glycerol-preserved amnion & fresh amnion on skin in terms of decreasing bacterial load in infected rat burn wounds. Amnion stored in glycerol was reliable and viable for a long period of time. Amnion banking could provide a vast quantity of AM for biologic dressing for burn treatment at very low cost, an element that's particularly important in developing countries.

Rinastiti M, Harijadi, Santoso AL, Sosroseno W (2006)[27] assessed histologically human amniotic membrane transplantation on rabbit's gingival wound. Study showed rapid epithelialization with suppressed inflammation, suggesting that AM transplantation may promote rapid gingival wound healing in rabbits compared to secondary healing.

Goulart MG, Teixeira RT, Rangel DC, Filho WN et al (2008)[28] evaluated the consequences of the homogenous amniotic membrane (AM) as a biological dressing material for oral mucositis induced by 5fluorouracil (5-FU) within the labial fornix region of inferior incisors in rats. Results were suggestive that HAM has more adhesive power when applied to the ulcerated surface, increasing the healing process and exhibited anti-inflammatory activity. Thus, HAM can be used as a biological dressing in treatment and management of oral mucositis in patients with chemotherapy and/or radiation therapy.

Kesting MR, Loeffelbein DJ, Classen M, Huspenina JS et al (2010)[29] showed successful closure of oronasal fistulas in minipigs using interposed grafts of cryopreserved HAM, which showed promising result as it is simple, cheap and effective technique for tension-free closure of such fistulas.

Peirovi H, Rezvani N, Hajinasrollah M, Mohammadi SS et al (2012)[30] assessed the feasibility of constructing a vein conduit from the amniotic membrane and implanting in the external jugular vein of juvenile sheep. At 5 weeks after implantation, the grafts was completely patent & displayed no signs of dilation or thrombus formation. After 48 weeks, grafts was still totally patent and displayed no signs of intimal thickening, dilation, or stenosis. No inflammation or fibrosis was

seen. Scanning electron microscopy showed a confluent layer of cells with normal endothelial cells. Thus confirming that the amniotic membrane can be a proper substitute for vascular tissue engineering.

Kim KS, Kim HS, Park JM, Kim HW et al (2013)[31] examined human placenta amniotic membrane-derived mesenchymal stem cells (AMSCs), which have potent immunomodulatory and paracrine effects on Tg2576 (APPswe) transgenic mouse model of AD. AMSCs secrete high levels of transforming growth factor-β under in vitro inflammatory environment conditions. The lower level of pro inflammatory cytokines, interleukin-1 and tumor necrosis factor-α was observed and level was higher of anti-inflammatory cytokines, interleukin-10 and transforming growth factor-β in AMSC-injected mice than phosphate-buffered saline-injected mice, indicating that injection of AMSCs might show significant long-lasting improvement in AD pathology and memory function via immunomodulatory and paracrine mechanisms.

Niknejad H, Yazdanpanah G (2013)[32] hypothesized that denuded AM (without epithelial cells) can induce angiogenesis. Result was observed on dorsal skinfold chamber method in an animal model and aortic ring assay showed that the epithelial side of the AM inhibits angiogenesis and reduced capillary numbers, while the mesenchymal side of the AM aggravate angiogenesis. This observation provide answer to the controversies about the angiogenic capability of the AM and raised some questions about the mechanisms by which the AM affects angiogenesis.

Cargnoni A, Piccinelli EC, Ressel L, Rossi D et al (2014)[33] demonstrated that injection of conditioned medium (CM) generated from cells of the mesenchymal region of human amniotic membrane (AMTCs) reduces bleomycin-induced lung

fibrosis in mice. AMTC-CM prevented lung fibrosis in bleomycin-challenged mice, improving survival rate and preserving lung functional parameters such as blood gas exchanges. The specificity of AMTC-CM action was observed by the absence of fibrosis reduction when other types of CM were used.

Tuncel U, Kostakoglu N, Turan A, Markoç F et al (2014)[34] evaluated the efficiency of temporalis muscle-fascia graft(tMFG), fresh and cryopreserved human amniotic membrane as an interpositional material in preventing temporomandibular joint ankylosis in a rabbit model. Conclusion was suggestive of interpositional arthroplasty with HAM and tMFG had similar effect in preventing TMJ ankylosis after discectomy in the rabbit model.

Yalniz-Akkaya Z, Ustun H, Ozkan Uney G et al (2014)[35] investigated the effect of subconjunctival amniotic membrane free graft on subconjunctival fibro vascular reaction. The number of fibroblasts, lymphocytes and macrophages was significantly higher within the Study Group than in Control Group. The number of Ki67- and SMA-positive cells, and CD34-positive vessels was also significantly higher within the Study Group. Free human amniotic membrane grafting without suturing found not useful in decreasing the subconjunctival fibro vascular reaction.

Fesli A, Sari A, Yilmaz N, Comelekoglu U et al (2014)[36] studied the effects of solitary usage and combined usage of amniotic membrane (AM) wrapping and granulocyte-colony-stimulating factor (GCSF) injections after primary repairment of transected Wistar‘s sciatic nerves of rats. Finally concluded that both AM wrapping and G-CSF injection have a super added effect on nerves regeneration and this effect is further synergized by combined use.

STUDIES IN THE FIELD OF OPTHALMOLOGY

Kobayashi A, Shirao Y, Segawa Y, Higashide T, et al (2001)[37] reported a case of successful management of pterygium with multi-layer amniotic membrane graft (AMG) in a young XP patient. The pain induced by surgery and irritation disappeared within a few days, re-epithelialization over the AMG was completely seen in 2 weeks. In addition, multi-layer AMG seemed to be useful for protecting bare sclera and extraocular muscles from mechanical injury.

John T, et al (2002)[38] reported a new surgical technique to manage severe acute toxic epidermal necrolysis & observed that this treatment help in preserving normal ocular and eyelid surfaces and prevent chances of blindness.

Bouchard CS, John T (2004)[39] attempted to assess the indications and transplantation based. Successful outcome was derived by them as the healing of an epithelial defect, corneal or conjunctival.

Nakamura T, Sekiyama E, Takaoka M, Bentley AJ et al (2008)[40] evaluated the efficacy and safety of trehalosetreated freeze-dried amniotic membrane (TT-FDAM) for ocular surface reconstruction & found TT-FDAM retained most of the physical, biological, and morphological characteristics of native AM, making it a useful biomaterial for ocular surface reconstruction.

Said DG, Nubile M, Alomar T, Hopkinson A et al (2009)[41] evaluated the histologic changes occurring in the transplanted amniotic membrane in human eyes. The epithelial cell migration and adhesion properties was shown by amniotic basement membrane. The amniotic stroma supports epithelial cells & CSDCs. Repopulation of

the amniotic stroma by CSDCs integrates the amnion with corneal tissue and allows rebuilding of corneal stroma.

Kassem RR, Gawdat GI, Zedan RH (2010)[42] used lyophilized amniotic membrane to wrap the extraocular muscles. Extensive adhesions observed with inelastic, fibrotic muscles at subsequent operation.

STUDIES IN FIELD OF BURNS

Ramakrishnan KM, Jayaraman V (1997)[43] used human amniotic membrane obtained from placenta of HbSAg, HIV-seronegative mothers undergoing caesarean section as a temporary biological dressing on superficial and deep partial-thickness burns. The advantages of amniotic membrane cover were reduction in pain, early drying of the wound and epithelialization. Due to the patient acceptability, reduction in duration of hospital stay and reduced cost, they concluded that treatment of superficial and deep partialthickness burns with amniotic membrane is ideal for a developing country.

Ravishanker R, Bath AS, Roy R (2003)[44] illustrated that long term glycerol preserved amniotic membranes are extremely economical for dressing and emphasized the importance for establishing such "Amnion Banks" in all hospitals especially in developing countries.

Singh R, Chacharkar MP (2011)[45] assessed the functional as well as clinical efficacy of air-dried radiation sterilized amniotic membranes as dressing in burns wound. Storage has no impact on the impermeability of the processed amniotic membranes to bacterial load. Thus dried gamma-irradiated amniotic membranes even

after 5 years of storage provided an effective barrier to microbial penetration. There were no qualitative changes within the material property of dried gamma-irradiated amniotic membranes even after 2 and 5 years of storage. Air-dried amniotic membrane storage at room temperature is an ideal dressing for burn wound care.

Gibert MA, Fauste SP (2012)[46] assessed the use of amniotic membrane transplantation in the treatment modalities of refractory chronic leg ulcers. Complete wound re-epithelialization was achieved with the corresponding reduction in pain intensity. No adverse effects was seen. They concluded that amniotic membrane transplantation could also be an effecient alternative for treatment of refractory chronic vascular ulcers on the lower limbs.

Mohammadi AA, Jafari SM, Kiasat M, Tavakkolian AR et al (2013)[47] illustrated that human amniotic membrane dressing on chronic infected wound, can significantly improve the success rate of graft taken due to its interesting antimicrobial effects.

STUDIES IN ORAL & MAXILLOFACIAL REGION

Lawson VG (1985)[48] used amniotic membrane with pectoralis major muscle at its deeper aspects for oral cavity reconstruction. Finally reached conclusion that amnion enhanced epithelialization of the oral mucosa and reduced scarring in the moderate-sized defects.

Lai, Chen HR, Lin LM, Huang YL et al(1995)[49] studied various modalities in the treatment of OSMF, one of which was the application of a monolayer of fresh amnion graft over buccal mucosa after the excision of the fibrotic bands. They discover that there was decrease in interincisal distance in the range of 5– 10 mm after 2 years of

follow-up & in 62% of cases with a fresh amnion graft , in comparison to 50% with a split thickness skin graft and 38% with use of a buccal fat pad graft. They proposed that fresh amnion graft was not effective in a mono layer over deeper buccal defects.

Hao SP (2000)[50] observed 21 patients undergone surgery with pedicled buccal fat pad flap in the reconstruction of oral defects. Out of 21, 1(5%) patients lend in to failure and one (5%) showed complication.

Ti SE, Tow SL, Chee SP (2001)[51] illustrated the role of amniotic membrane transplantation for the management of cicatricial eyelid entropion. A gray line lid split procedure with vertical anterior lamella repositioning were performed on patients with moderate to severe cicatricial entropion. The AM accelerated the epithelialization of bare tarsus; this was observed due to lack of fluorescein staining and reversion to skin color within 2 to 3 weeks. However, AM could not prevent tarsal shrinkage. Study concluded the use of AM in a lid split procedure for correction of cicatricial entropion helps in bare tarsus epithelialization rapidly as well as improves the initial cosmetic result of surgery.

Mehrotra D, **Pradhan R, Gupta S (2009)[52]** this study showed comparison between the tongue flap, buccal fat pad flap, nasolabial flap, and split thickness skin graft for the coverage of mucosal defects after excision of fibrotic bands in OSMF. They noticed no statistically significant difference for oral opening, but in terms of total score for pain, aesthetics, and function after 1 month was more in the buccal fat pad group suggestive of better results.

Safawi EB, Halim AS, Khoo TL, Dorai AA (2010)[53] illustrated 7 years of work with dried irradiated human amnion in the treatment of facial burns. No patients developed

facial wound infections. 85 % of the patients required only a single application of the dried amnion. The average healing time was 5.4 days. Thus, concluded that superficial partial thickness facial burns can be effectively and efficiently treated with dried irradiated human amnion membrane.

Amemiya T, Nakamura T, Yamamoto T, Kinoshita S et al (2010)[6] developed a novel method for cultivation of oral mucosal epithelial cell sheets. Specimens of AM collected from women undergoing Caesarean section. Using oral mucosal biopsy specimens obtained from the patients, oral epithelial cells were cultivated on an AM carrier. Thus, resultant sheet than was transplanted on the oral mucosal defect. After 2-3 weeks of culture, the cultivated epithelial cells were well differentiated and showed stratification into 5-7 layers on AM. Immunohistochemistry demonstrated that the cultivated cells expressed highly specific mucosal epithelial cell markers and basement membrane proteins. After the surgical procedure, the reconstructed sites did not show any features of infection, bleeding, rejection, or sheet detachment, and the sites achieved a new oral mucous membrane. The cultivated epithelial sheets maintained the properties of a mucous membrane and expressed basement membrane proteins. 12 months follow up showed the postoperative period were uneventful. These findings concluded that, this novel epithelial sheet is a useful biomaterial for mucosal reconstruction procedures.

Lo V, Lara-Corrales I, Stuparich A, Pope E (2010)[54] illustrated the potential efficacy of amniotic membrane as grafting material in promoting healing in patients with epidermolysis bullosa and chronic, non-healing wounds.

Chuan HY, Lee HW, Chen YT, Young TH et al (2011)[55] illustrated the effects as well as the underlying mechanism of AM on salivary gland morphogenesis. On AM stromal scaffold, SMG showed well-developed branching morphogenesis. On AM epithelial scaffold, SMG epithelial cell was converted to a spindle-shape, with loss of intercellular connection, modified cytoskeletal organization, and exhibited scattering behaviors. However, when acellular AM epithelial scaffold was used, cultured SMG showed organized morphology, pointing that AM epithelial component provided specific surface features for SMG morphogenesis. After blocking HGF function of AM, cultured SMG regained branching activity, reorganized cell adhesion and subcellular organization, and reproduced basement membranes.

Manjunath Rai, Ramaraj PN, Sharma A (2011)[56] studied the Amniotic Membrane uses as a Dressing material in Cervical Necrotizing Fasciitis and concluded that the AM when used as a temporary dressing over these wounds increases the healing rate and granulation tissue formation, making the recipient defect more apt to accept a secondary reconstructive procedure if required. Use of the AM provides a novel approach to prevent the need for extensive flaps or grafts to close the post debridement defect.

Arai N, Tsuno H, Okabe M, Yoshida T et al (2012)[57] evaluated the usefulness of a hyper-dry amniotic membrane (AM), a novel preservable human amnion, used as a wound-dressing material for surgical defects of the oral mucosa. They proposed that hyper-dry AM is biologically acceptable as a dressing materials for oral wounds and should be a suitable clinical alternative for the repair of the oral mucosa.

Tsuno H, Arai N, Sakai C, Okabe M et al (2012)[58] reported 2 cases with intraoral alveolar wounds with bone exposure which was successfully treated with the use of hyper-dry amniotic membrane. Thus concluded that the hyper-dry amniotic membrane is a useful dressing material, not only for soft tissue wounds but also used for exposed bone in the oral cavity.

Khademi B, Bahranifard H, Azarpira N, Behboodi E (2013)[59] evaluated the effectiveness of AM as a biological agent for wound as a dressing material for surgical defects of mucosa in the oropharyngeal region. In this study 50 patients with primary oropharyngeal malignancy who underwent tumor resection were enrolled. They used amniotic membrane in dressing of the defects in the oral cavity and pharynx. Efficacy of this was assessed by rating of the pain and granulation tissue formation with surface epithelialization at the site of graft. Finally concluded that amniotic membrane may be used as a biologic dressing material for covering the mucosal defects in the oropharynx.

Singh H, Singh H (2013)[60] reported a case of isolated gingival recession in patient treated successfully using coronally advanced flap with amnion membrane, used as guided tissue regeneration (GTR) membrane. Hence the site clinically showed significant root coverage with uneventful healing.

Shimane T, Shimane T, Aizawa H, Li Y et al (2014)[61] observed that a clinical need exists for an immunologically compatible surgical patch for soft tissue replacement, body wall repair, and as a wound dressing. Amniotic membrane is a light-weighted, thin and elastic in nature and it can promote cell attachment, proliferation and differentiation. Hyper-dry amniotic membrane can be preserved at room temperature

and used to culture epithelial cell for transplantation. In their study, they made human oral mucosal equivalent using hyper-dry amniotic membrane as a matrix.

Honjo KI, Amemiya T, Adachi K, Nishigaki M, et al (2014)[62] revealed that the amniotic membrane (AM) is a thin membrane which is the outermost layer of the placenta and composed of parenchyma of a constant thickness. It is usually collected from the placenta and obtained without any ethical or technical problem as it is generally discarded after delivery. It has been in use for various surgeries, for its usefulness and effectiveness as a culture substrate as well as graft material. They focused on usefulness of AM and prepared various cultured cell sheets (oral mucosal epithelial, periodontal ligament-derived, and pulp-derived cell sheets) using the membrane as a substrate.

I. B. Kar, Singh AK, Mohapatra PC, Mohanty PK et al(2014)[63] this prospective study aim to evaluate the clinical outcome of the surgical repair of oral mucosal defects using cryopreserved human amniotic membrane (HAM) as a graft material. Thirtyfour patients were included with precancerous lesions such as leukoplakia, erythroplakia, and verrucous hyperplasia. Fresh amniotic membrane were obtained from the placenta of women undergoing elective caesarean section; the membrane was than cleaned, prepared in antibiotic solutions, and preserved at -80 ^{0}C. Results suggested that HAM elevate healing and epithelialization without specific complications. Finally concluded that the use of HAM gives exellent results in the repair of post-surgical oral mucosal defects.

Subha Lakshmi, Bharani S, Ambardar K(2015)[64] used a human amniotic membrane to repair an iatrogenic oroantral communication that occurred during the

extraction of right upper second molar of the patients. A splint was given after the perforated area was covered with human amniotic membrane and healing was clinically assessed at various time intervals. The outcome of the study revealed that the human amniotic membrane was an efficient graft material for repairing the defect caused by an iatrogenic oroantral communication following tooth extraction.

Takeshi Amemiya Nakamura T, Yamamoto T, Kinoshita S et al(2015)[65] investigated the autologous transplantation of oral mucosal epithelial cells cultured on AM in patients undergoing oral surgeries. Five patients were included in the study that underwent autologous cultured oral epithelial transplantation following oral surgical procedures. Patients were followed-up for a minimum of 12 months after transplantation. After surgical procedures no evidence of infection, bleeding, rejection, or sheet detachment seen on reconstructed sites. Hence suggested that AM-cultured oral mucosal epithelial cell sheets represents as a useful biomaterial and feasible method for oral mucosal reconstruction.

Chakrawarti S, Aurora JK, Bedi RS, Shiva Mani et al(2019)[66] did a prospective study to assess the versatility of Human Amniotic Membrane (HAM). 15 patients has been enrolled which have post surgical soft tissue defect requiring primary wound coverage application with HAM. Patients has been assessed on subjective (pain and sensory response) and objective parameters (swelling, mouth opening, epithelization, mucosal suppleness & scar contracture) over a period of more than 6 months. Results manifested remarkable improvement in pain. In OSMF cases, mouth opening was significantly increased in comparision to pre-op mouth opening. Swelling was found to decrease on 7^{th} post op day. The entire patient showed good sensory response (100%). Epithelization was good while one patient presented with reoccurrence.

Mucosal suppleness and scar contracture was good in 93.3% patients while only 6.7% patient presented with poor mucosal suppleness and scar contracture. Clinical acceptability and applicability of HAM with diverse properties makes it a flexible allograft material in maxillofacial reconstruction of soft tissue defects.

TMJ

Bauer F, Hingsammer LM, Wolff KD, Kesting MR **(2013)**[67] reported the usage of human amniotic membrane in combination with a costochondral graft as an interpositional material in temporomandibular joint reconstruction surgery for the first time. Because of the very fact that currently used interpositional materials does not prevent the recurrence of temporomandibular joint ankylosis sufficiently, they demonstrated anti adhesive properties of amniotic membrane and highlighting its multifaceted use in various fields.

VESTIBULOPLASTY

Guler, Ercan MT, Ulutuncel N, Devrim H et al(1997)[68] reported the use of amnion in vestibuloplasty, which focuses on collecting data on blood flow to the graft. Thus, concluded that amnion graft may be better alternative to other graft materials in mandibular vestibuloplasty because of faster healing, and observed the angiogenic function of the amnion start within first 10–15 days and improve to the normal blood flow postoperatively by day 30.

Samandari MH, Yaghmaei M, Ejlali M, Moshref M_ et al (2004)[5] evaluated the use of amnion as a biodegradable graft material for vestibuloplasty. A white necrotic soft tissue layer was seen underlying hyperemic tissue and an average reduction of 1

to 3 mm in the depth of the labial vestibule was seen after a week. By the end of the 2nd week, the necrotic layer disappear, leaving some hyperemic mucosal tissue. By the 3rd week, the graft area was not notifiable as amnion had completely degenerated and disappeared. The reduction in the depth of the buccal vestibule ranges between 17% to 40% after 6 months follow-up. Amnion might be potential graft material for vestibuloplasty.

Kothari CR, Goudar G, Hallur N, Sikkerimath B et al (2012)[69] showed the clinical efficacy of amnion membrane as a graft material for vestibuloplasty & for increasing the depth of the sulcus for complete rehabilitation with dentures. Thus concluded that amniotic membrane graft are viable and reliable for covering of the raw surface & help to prevent secondary contraction after vestibuloplasty, and maintain the vestibular depth postoperatively.

OTHER RECONSTRUCTIVE USES

Mhaskar R (2005)[70] showed the use of amniotic membrane grafts in surgeries of cervical and vaginal agenesis. Study was conducted on 5 girls with complete cervical and vaginal agenesis who underwent cervicoplasty and vaginoplasty with the use of amniotic membrane grafts. Excellent epithelization and patency of cervix and vagina was achieved in almost all cases. This showed that Amniotic membrane can be used as an allograft in cervical reconstruction. It was cost effective, readily available, of low antigenicity and does not require repeated cervical dilatation. This was the first case series reported in the literature which showed use of amniotic membrane for cervical reconstruction.

Shojaku H, Takakura H, Okabe M, Fujisaka M et al(2011)[71] in this study approach was to investigate usefulness of human AM patches in canal wall down tympanoplasty as a substitute graft. Almost every patients showed complete epithelialization of the mastoid cavity and faster rate of epithelialization with AM graft as compared with the fascia graft.

Iravani K, Hashemi SB, Tehrani M, Rashidi M (2014)[72] has reported a case of chondrosarcoma(low grade) of the larynx, which was surgically treated with use of both amniotic membrane and stent in airway reconstruction procedure following laryngofissure approach for tumor resection.

BUCCAL FAT PAD

Khanna J N and Andrade N N (1995)[73] evaluated the surgical management of OSMF.A series of 100 cases of OSMF were included and divided into four groups. Group I (Very early stage without mouth opening limitations with an interincisal distance of greater than 35 mm) and Group II (Early stage with interincisal distance of 26-35 mm) (25 cases), were treated by local injections. Group III (Moderately advanced cases with an interincisal distance of 15-25 mm) and group IV (Severe trismus with an interincisal distance of 15 mm) (75cases) were treated surgically. Coverage of mucosal defect with split –thickness skin grafting, along with temporalis myotomy and bilateral coronoidectomy was the treatment protocol in group III and IV.

Lai DR, Chen HR, Lin LM, Huang YL, et al (1995)[74] evaluated different effective regimen for the treatment for OSMF . The treatment of surgical group was done by excision of fibrotic tissue and covering of the defect with split thickness skin, fresh

human amnion or buccal pad fat grafts. The treatment was selected on the basis of stages with clinical progression to gain maximal interincisal distance. The surgical therapy results in significant improvement of trismus in cases of severe impairment (Inter incisal distance < 20mm). The authors concluded that buccal pad fat grafting was particularly successful in reducing scarring, as compared to the other flaps taken from radial forearm and nasolabial.

Yeh CJ (1996)[75] performed a prospective study on the application of buccal fat pad in the treatment of patients suffering from trismus caused by oral submucous fibrosis. According to the authors buccal fat pad has been widely used for the repair of oral defects. The patients underwent excision of the fibrotic bands and coverage of the buccal defect with a pedicled buccal fat pad flap.

Chiao KC, Chang LC, Liu SY, Wang JJ (2002)[76]observed the wound healing process microscopically of buccal fat pad (BFP) grafted on a defect of the buccal mucosa in OSMF. The defects created were then covered by a BFP graft. The result showed inflammatory cell infiltrates, blood vessel congestion, and fibrinous exudates covering the BFP were obvious and the number of fat cells decreased significantly. The original BFP was almost completely replaced by granulation tissue and covered by stratified squamous epithelium by week 5. The authors concluded that BFP graft can be widely used for covering exposed defects created by fibrotic band excision for the improvement of mouth opening limitation. The healing process was documented microscopically by weekly observations.

Adeyemo WL, Ladeinde AL, Ogunlewe MO, Bamgbose B O(2004)[77]reviewed the efficacy of buccal fat pad (BFP) in oral reconstruction, 25 years after its first use as a

pedicled flap. They found that the various application of BFP in oral reconstruction surgeries including closure of surgical defects following tumor excision, repair of surgical defects following excision of leukoplakia and submucous fibrosis, closure of primary and secondary palatal clefts, coverage of maxillary and mandibular bone grafts and lining of sinus surface of maxillary sinus bone graft in sinus lift procedure for maxillary augmentation. They concluded that easy mobilisation of the BFP and its excellent blood supply, and minimal donor site morbidity makes it an ideal flap the main advantages of BFP include ease of harvesting, simplicity, versatility, low rate of complications as well as less operating time. .

Shah A, Raj S, Rasaniya V, Patel S, et al (2005)[78] evaluated the efficacy of surgical excision of fibrotic bands with laser without any graft to cover wounds, and to maintain mouth opening postoperatively by physiotherapy. A study was conducted using an Opus diode laser to relieve trismus caused by OSMF. Ten patients diagnosed clinically with oral submucous fibrosis involving buccal mucosa, retromolar pad area were treated by releasing fibrotic bands with laser. Patients preoperative spontaneous mouth opening ranged from 16 to 24 mm. Result showed average increase of mouth opening was 15.0 mm in group III (moderately advanced cases) and 17.0 mm in group II (early cases)over a follow-up period of 3 months. The average mouth opening was 32 mm and no wound dehiscene was noted in any patient. The authors concluded that treatment for oral submucous fibrosis using laser is a simple surgical procedure with effective results.

Talsania JR, Umakant, Shah UB, Shah AI, et al (2006)[79] evaluated the efficacy of laser to reduce trismus in OSMF. A total of 8 patients, 5 (62.5%) in the age group of 21–30 years, 2 (25%) in the age group of 31–40 years and 1 (12.5%) in the age group

of 11–20 years were included in the study. Results showed that laser therapy eliminates the use of grafts to close the surgical defect inspite of extensive resection. It yields excellent cosmetic and functional results, and patients were relieved of trismus in all cases with mean postoperative interincisal distance of about 33.25 mm. Authors concluded that Diode laser gave good results, and is a less expensive and alternative method in group III and group IV cases.

Gnanam A., Kamal K., Venkatachalapathy S., Jasline D. (2010)[80] evaluated various surgical treatment modalities for oral submucous fibrosis and their outcome. Patients underwent surgical excision of the fibrous bands and reconstruction of the surgical defect by flaps such as buccal fat pad. The resultant postoperative mean mouth opening was 40 mm found to be improved in all the patients in about three weeks with oral physiotherapy compared to preoperative mouth opening 20 mm. They concluded that grafting the defect with a graft is useful in the management of oral submucous fibrosis, and grafting with buccal pad of fat was found to be reliable.

Sharma R, Thapliyal GK, Sinha R, Menon S (2011)[81] evaluated clinically the application of pedicled buccal fat pad (BFP) in the surgical management of stage III and IV oral submucous fibrosis (OSMF). 28 clinically and histologically Confirmed case of OSMF were taken and divided into 2 groups: group I (n15) and group II (n13), corresponding to clinical stage III and stage IV. The result showed that mean preoperative mouth opening was 19.6 mm in group I and 12.92 mm in group II. The mean postoperative mouth opening after 1 year was 35 mm in group I and 31.76 mm in group. They concluded that BFP was reliable for the surgical treatment of OSMF.

Kothari MC,Hallur N, Sikkerimath B, Gudi S, et al (2012)[82] evaluated coronoidectomy, masticatory myotomy and buccal fat pad graft in advanced (Stage III–IV) oral submucous fibrosis. 10 advanced OSF cases which were clinically and histologically confirmed underwent surgery entailing bilateral coronoidectomy, masticatory muscle myotomy and closure with a pedicled buccal fat pad graft followed by vigorous mouth opening exercises. Results showed a mean interincisal opening of 14.7 mm preoperatively and 32.5 mm at 12 months postoperatively. Authors concluded that the regime was successful and effective in the management of OSMF.

Saravanan K, Narayanan V (2012)[83] evaluated in the study about the buccal fat pad use in surgical management of oral sub mucous fibrosis as an interpositioning material. Pedicled buccal fat pad used as an interpositioning material to cover the raw areas in the oral cavity after incision and release of fibrous bands. Resulted in the successful surgical procedure, the size of the intra operative mouth opening was ranged from 25–38mm. Authors concluded BFP seems to be an appropriate interpositional graft in the surgical management of OSMF.

Pradhan H, Gupta H, Sinha VP, Gupta S et al (2012)[84] evaluated the effectiveness of coronoidectomy in advanced (stage III-IV) oral submucous fibrosis (OSMF) Five patients clinically diagnosed as grade III/IV OSMF underwent surgery entailing coronoidectomy in addition to conventional surgical procedures followed by vigorous mouth opening exercises. Authors concluded coronoidectomy is an effective adjunct in increasing intra operative and stabilizing postoperative mouth opening.

Prashanth R, Nandini GD, Balakrishna R (2013)[85] evaluated the versatility and effectivity of pedicled buccal fat pad in the reconstruction surgery of intra oral defects. The Postoperative follow up was followed at interval of 1st, 7th and 15th day, at 1 month, 2nd month and 3rd month was performed. Results showed procedure was successful in all the patients. Post operatively healing was satisfactory with no breakdown or liquefaction necrosis. All the patients had definitive colour change at the end of 1st postoperative month owing to the epithelialization.

Rai A, Rai M, Datarkar A(2013)[86] compared the buccal fat pad (BFP) and nasolabial flap for reconstruction of intraoral defects after release of fibrous bands in patients with oral submucous fibrosis (OSF). 20 patients were divided into two groups. In Group I (n=10), reconstruction was performed using a nasolabial flap and in Group II (n= 10) with BFP. In 20 patients, interincisal mouth opening was (mean) 11 mm preoperatively which improved to a mean of 42 mm. In Group I complication were more as compared to Group II such as partial flap necrosis particularly at the tips, temporary widening of oral commissure and subluxation of TMJ. The unsightly extraoral scar. The authors concluded that BFP is a better choice for reconstruction in comparison to nasolabial flaps.

Gupta H, Tandon P, Kumar D, Sinha VP et al. (2012)[87] evaluated the effectiveness of coronoidectomy in advanced (stage III-IV) oral submucous fibrosis (OSMF) and temporomandibular joint (TMJ) ankylosis. Five grade III/IV OSMF patients who were clinically diagnosed placed in group 1 and seven TMJ ankylosis patients who were clinically and radiographically confirmed were placed in group 2 underwent surgery entailing coronoidectomy in addition with conventional surgical procedures used in both conditions followed by vigorous mouth opening exercises.

The results was evaluated using the interincisal distance at maximum mouth opening as the objective outcome over a follow-up period of 2 months. OSMF patients (group I) showed a mean preoperative interincisal opening of 14.40 mm which was increased further by the use of conventional procedures to 24.60 mm and showed further increment after unilateral and bilateral coronoidectomy to 35 and 44.80 mm. In TMJ ankylosis patients (group II), preoperative mean mouth opening of 6.71 mm increased further by the use of conventional procedures to 24.29 mm, and further to 37.29 mm after unilateral coronoidectomy. Authors concluded coronoidectomy is an effective adjunct in increasing intra operative and stabilizing postoperative mouth opening.

Naphande M, Adwani D, Bhagat B, Quraishi AQ et al(2014)[88] Studied a case of OSMF of age 26 years female patient with initial interincisal mouth opening of 10 mm which was treated surgically with buccal fat pad flap reconstruction technique followed by active mouth opening exercise for 6 months. Result showed with maintained increased 26 mm interincisal mouth opening and was followed up to 1month. Authors concluded Buccal fat pad technique in the management of OSMF with postoperative exercise played important role in gaining functional disease free mouth opening

Lambade P., Dawane P., Thorat A. (2016)[89] evaluates the effectiveness of buccal fat pad in oral submucous fibrosis treatment. 20 patients were enrolled & treated for oral submucous fibrosis with interincisal mouth opening less than 16 mm. Surgical procedure comprises of fibrotomy, all third molar extractions, and coronoidotomy or coronoidectomy followed by reconstruction of fibrotomy defect with buccal pad fat. Result showed excellent increase in the interincisal mouth opening relieving trismus. Buccal fat pad underwent rapid epithelization within a period of 5-7 weeks. Authors

concluded Buccal fat pad can beused effectively in the surgical management of oral submucous fibrosis with good functional and esthetic outcome.

Wahab N, Razi A, Iqbal A, Ali H et al (2016)[90] evaluated the clinical and anatomical application of buccal fat pad as an interpositional material in the surgical treatment of oral submucous fibrosis (OSMF) for improvement in mouth opening and its movements. Better clinical improvement was seen with minimal morbidity by the use of buccal fat pad in patients with severe limited mouth opening along with regular and vigorous physiotherapy. Comparison was performed at interval like pre-operative with intra and post-operative mouth opening came out to be statistically significant. Thus, concluded that use of interpositioning buccal fat pad flap improves the mouth opening and movement in OSMF patients.

Patil SB, Durairaj D, Kumar GS, Karthikeyan D et al (2017)[91] assessed the comparison between the application of extended nasolabial flap & buccal fat pad graft for the surgical management of oral submucous fibrosis. Authors concluded that buccal fat pad graft was far superior & gave better results as the interposition material due to rapid epithelization, minimal donor site morbidity, good patient acceptance and minimal intra and postoperative complications.

MATERIALS AND METHODS

Study design

Patient reported to the OPD of Oral and Maxillofacial Surgery, Babu Banarasi Das College Of Dental Sciences, Lucknow, with Grade III or Grade IV Oral Submucous Fibrosis (OSMF) was included in the study

Patient were divided into 2 groups based on two surgical site(left side and right side)

Diagnosed patients with OSMF were divided into two surgical site (n=5 patients)

Group I - Left side buccal mucosa in which resection of fibrous band with coronoidectomy followed by reconstruction of the mucosal defect with BFP.

Group II - Right side buccal mucosa in which resection of fibrous band with coronoidectomy followed by reconstruction of the mucosal defect with Freeze dried irradiated Amniotic Membrane.

Preoperative assessment included a thorough history and physical examination, measurement of maximal incisor opening (MIO), presence or absence of 3rd molars, extent & severity of fibrosis and causative factors / deleterious habits.

Inclusion criteria-

(Patients for surgery were selected irrespective of sex, religion or socioeconomic status on the basis of the following criteria)

- Grade III and Grade IV-A OSMF according to Khanna and Andrade grading requiring surgical intervention.

- Patient who has given their written informed consent for the study.
- Medically fit patient for surgery under general anesthesia.
- Above >18 years of age patients were included.

Exclusion Criteria

- Patients with previous surgical treatment for oral submucous fibrosis.
- Grade I, Grade II OSMF according to Khanna and Andrade grading.
- Patients with any systemic diseases, which might have significance in wound healing.

Materials:

Armamentarium

- ✓ Mouth mirror and probe
- ✓ Tweezer
- ✓ Bard Parker handle- No. 3 and Blade- No. 15
- ✓ Metallic scale
- ✓ Heister
- ✓ Metzenbaum scissor
- ✓ Langenbeck retractor
- ✓ Howarth's Periosteal elevator
- ✓ Molt's Periosteal elevator
- ✓ Osteotome

- ✓ Chisel
- ✓ Surgical mallet
- ✓ Forked ramus retractor
- ✓ Kocher's forceps
- ✓ Adson tissue forceps
- ✓ Allis tissue forceps
- ✓ Needle holder
- ✓ 3-0 Vicryl , 3-0 black silk suture
- ✓ Heith's Suture cutting scissors
- ✓ Freeze dried irradiated Amniotic Membrane (Procured from TATA MEMORIAL HOSPITAL, MUMBAI)

Methods of collection of Data:

The patients under study were ASA Class I and relatively healthy ASA class II patients. Inter-incisal mouth opening and widening of commisure of mouth were measured pre-operatively, intra-operatively, 2nd day, 1week, 1 month, 3 months and 6 months post-operatively for all patients.

Methodology:

1. The preoperative and postoperative mouth opening in both the groups were evaluated in millimetres (mm).

2. The pre and postoperative oral commissural width was measured in mm.

3. The complications encountered during and after the surgery were studied.

After routine laboratory and radiological investigations and written consent, pre-anaesthetic evaluation were done. The patient was prepared as per the routine aseptic protocol, and under laryngoscope assisted intubation, general anaesthesia was administrated.

In Group I

Incisions was given by using bard parker blade no-15 extended from the corner of mouth to more posteriorly at a level of the linea alba, avoiding injury to stensons duct. Fibrotomy of the bands were done. The coronoid processes were approached through the same incision and a bilateral coronoidectomy was carried out. The maxillary and mandibular third molars were extracted bilaterally, interincisal opening was recorded. The Buccal Fat of Pad was harvested through the posterior superior margin of the buccal defect that created (raw area created after the fibrotomy procedure up to the retero molar trigone region). The average length of 3 cm and width of 4 cm was harvested depending on the size of the defect. The BFP was teased out gently until enough BFP is obtained to cover the raw area without tension. The flap was sutured to the defect with the help of interrupted and mattress sutures with 3-0 vicryl. The BFP was used to cover the entire defect.

In Group II

Incisions was given by using bard parker blade no-15 extended from the corner of mouth to more posteriorly at a level of the linea alba, avoiding injury to stensons duct. Fibrotomy of the bands was done. The coronoid processes were approached through the same incision and a bilateral coronoidectomy or were carried out. The maxillary and mandibular third molars were extracted bilaterally, interincisal opening was recorded. The freeze dried irradiated Amniotic Membrane was used to cover the

entire defect. The Membrane was sutured to the defect with the help of interrupted and mattress sutures with 3-0 vicryl.

Physiotherapy was started from the 5th postoperative day and patients was instructed to continue the physiotherapy themselves for upto 6 months to prevent relapse.

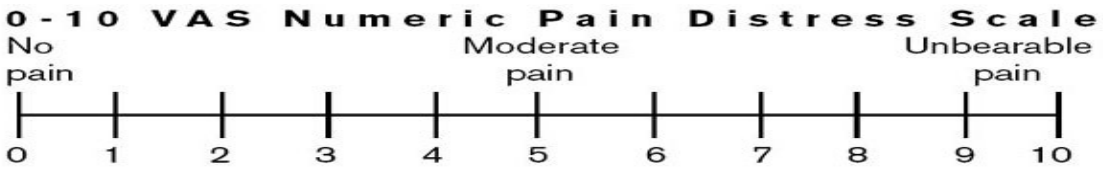
Parameter Assessment-

- Pre and post op. Mouth opening.
- Pain assessment- VAS (visual analog scale)
- -Swelling- preoperative and postoperative measurement.
- -Relief of symptoms :
- Burning sensation to hot and spicy food : yes or no (days elapsed since surgery)
- -Suppleness – suppleness was recorded by manual palpation by a single operator.
- Colour of the mucosa: White/Opaque, Pale, Normal.
- Healing:- Time till complete epithelization in days.
- Healing index as per Laundry et al –clinical assessment of the chain of events taking place during healing process of the defect was done on regular follow ups by a single observer.
- -Complication.

Parameters used are as follow:-

Pain assessment scale

The pain was assessed using VAS scale. The pain VAS is a continuous scale comprised of a horizontal (HVAS) or vertical (VVAS) line, usually 10 centimeters (10 mm) in length, anchored by 2 verbal descriptors, one for each symptom extreme. For pain intensity, the scale is most commonly anchored by "no pain" (score of 0) and "pain as bad as it could be" or "worst imaginable pain" (score of 10 [10-mm scale]). The pain VAS is self completed by the respondent. The respondent is asked to place a line perpendicular to the VAS line at the point that represents their pain intensity. Using a ruler, the score is determined by measuring the distance (mm) on the 10-cm line between the "no pain" anchor and the patient's mark, providing a range of scores from 0–10 (6). The pain VAS have been recommended: no pain (0–4 mm), mild pain (4-6mm), moderate pain (6–7 mm), and severe pain (7– 10 mm).



Swelling assessment

a) Preoperatively and postoperatively a line from tragus of ear to angle of mouth on the same side and the distance was measured using non elastic measuring tape. Preoperative measurements was subtracted from postoperative measurements to get the net inflammatory edema value.

b) More than 10% increase in postoperative value was considered as swelling and will be scored as present

c) Less than 10% increase in postoperative value was considered as swelling absent.

Suppleness

Implies to softness or elastic property of newly formed mucosa. Assessed by manual tactile sensation of the observers finger tips.

Score	
1	Stiff
2	Slightly stiff
3	Slightly supple
4	Supple

Healing index by Laundry et al.

1	Very poor	Tissue colour >50% of tissue red, Response of palpation-bleeding, Granulation tissue-present, Incision margin –not epithelialized with loss of epithelium beyond incision margin, suppuration present
2	Poor	Tissue colour >50% of tissue red, Response of palpation-bleeding, Granulation tissue-present, Incision margin-not epithelialized with connective tissue exposed
3	Good	Tissue colour >25% of tissue red, Response of palpation-bleeding, Granulation tissue-present, Incision margin-not epithelialized with connective tissue exposed
4	very good	Tissue colour >25% of tissue red, Response of palpation- no bleeding, Granulation tissue- present,

		Incision margin-not epithelialized with connective tissue exposed
5	Excellent	Tissue colour all pink Respose to palpation-no bleeding, Granulation tissue-none, Incision margin-not epithelialized with connective tissue exposed

Infection: Pus discharge.

	Absent	Present
Score	0	1

Pigmentation: Observed by a single observor.

	Hypopigmented	Normal	Hyperpigmented
Score	1	2	3

Analysis of parameters

Follow up were done from the day of operation upto 6 months. Assessment was done under following parameters on postoperative day 2 , 1 week,1 month , 3 months and 6 months:

- Appearance- Colour of mucosa-(1)white/opaque,(2) pale, (3)normal
- Pain-using visual analog scale-ask patient to rate the pain on a scale of 0-10, with 0 indicating no pain and 10 severe pain.
- Swelling-absent (0), present (1).
- Suppleness of mucosa-can be stiff (1), slightly stiff (2), slightly supple (3), supple (4)

- Infection – absent (0), present (1).
- Pigmentation –hypo (1), Normal (2), hyper (3).

All data were collected and tabulated and analyzed using appropriate statistical analysis.

PHOTOGRAPHS

ARMAMENTARIUM

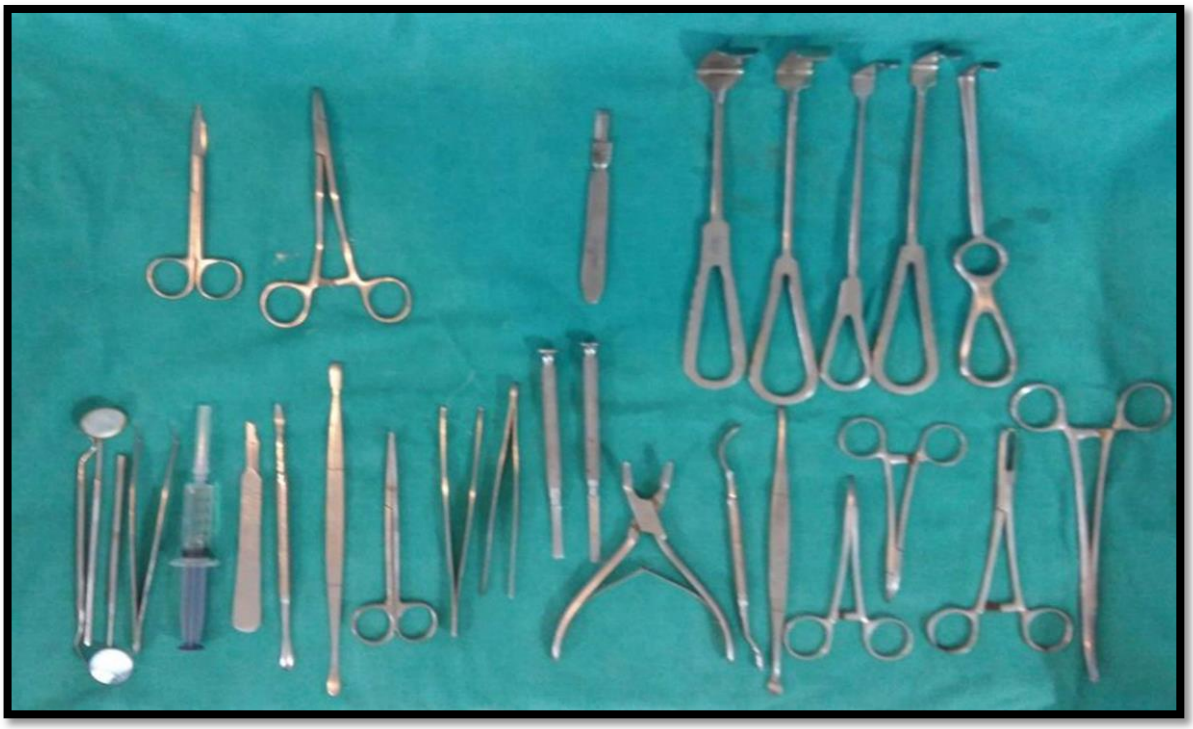


Fig 1: ARMAMENTARIUM REQUIRED FOR SUBMUCOUS FIBROSIS SURGERY

Fig 1.2: ARMAMENTARIUM REQUIRED FOR SUBMUCOUS FIBROSIS SURGERY

PATIENT PHOTOGRAPHS

PREOPERATIVE

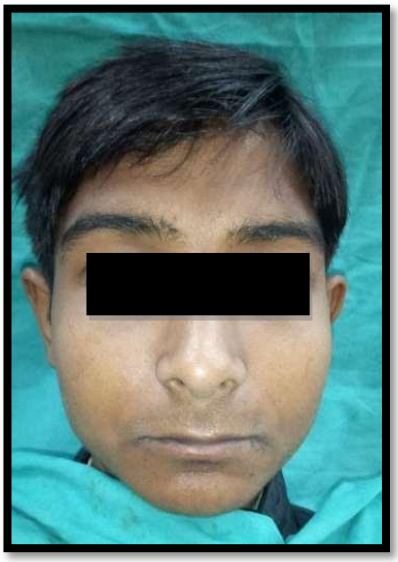


Fig 2.1: Frontal view

Fig2.2: Mouth Opening – 10 mm

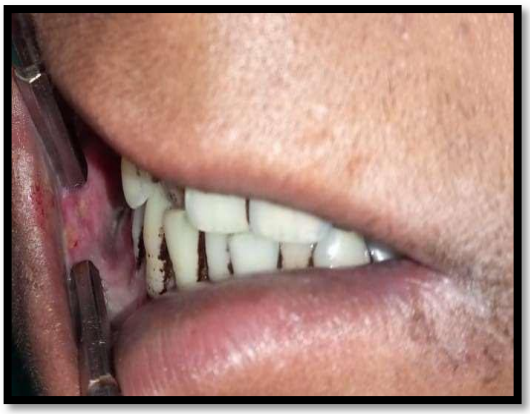


Fig 2.3: Right Side Buccal Mucosa

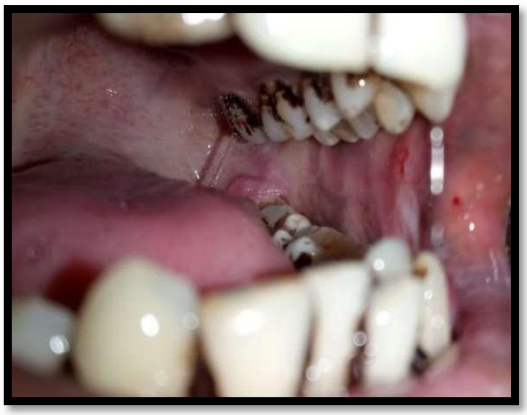


Fig 2.4: Left Side Buccal Mucosa

INTRAOPERATIVE

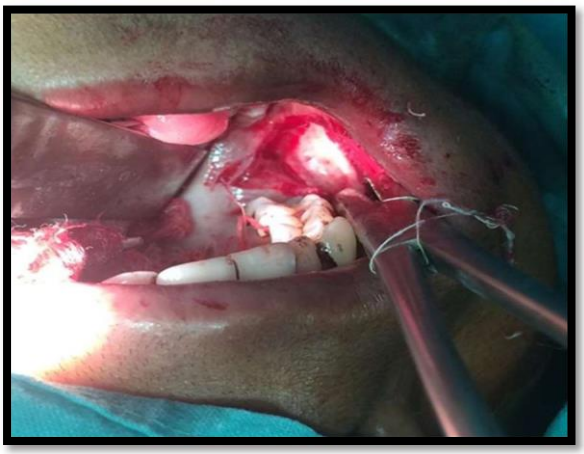


Fig 3.1: Resection of Fibrous Bands

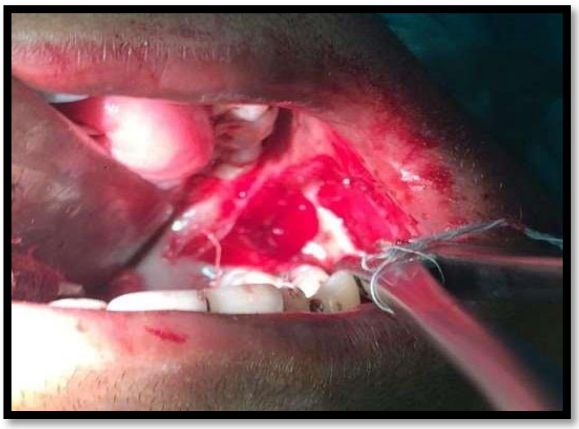


Fig 3.2: Masticator Muscle Myotomy

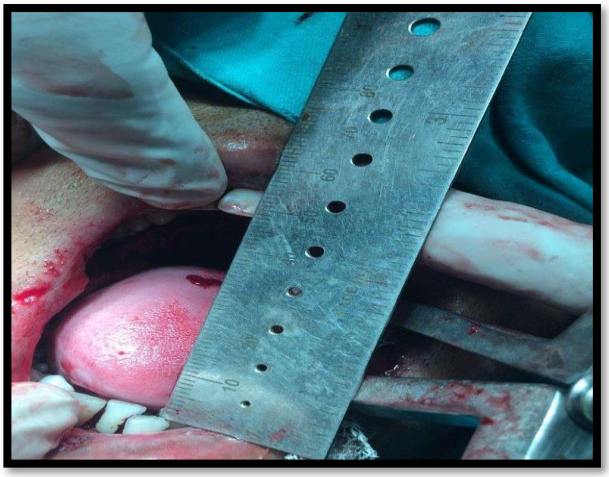


Fig 4: After Bilateral Resection of Fibrous Bands Mouth Opening Achieved 48mm

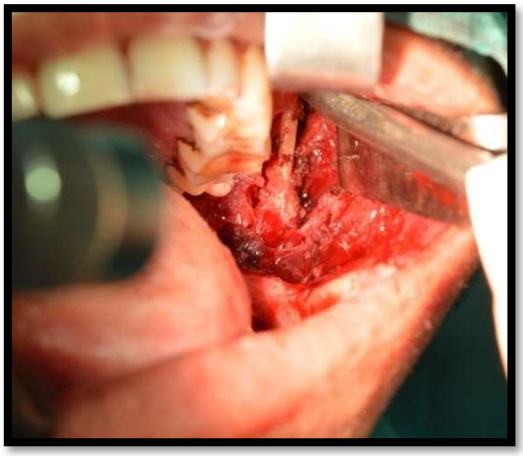


Fig 5: Exposure of coronoid

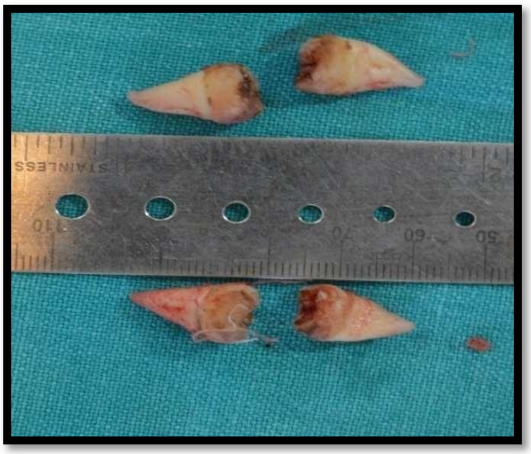


Fig 6: Extracted all 3rd Molars

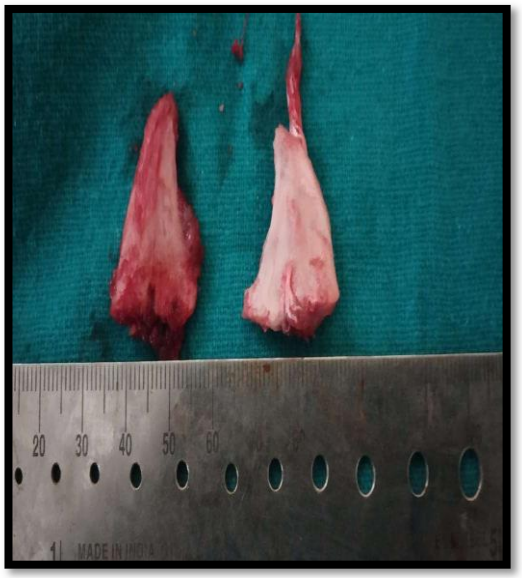


Fig 7: Removal of bilateral coronoids(Coronoidectomy)

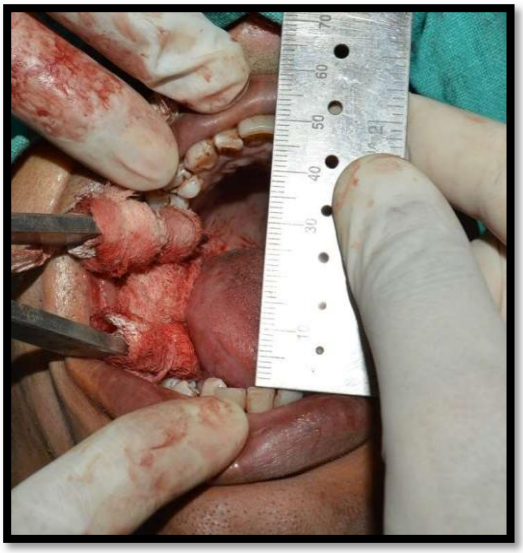


Fig 8: Bilateral Coronoidectomy Mouth opening Achieved 48

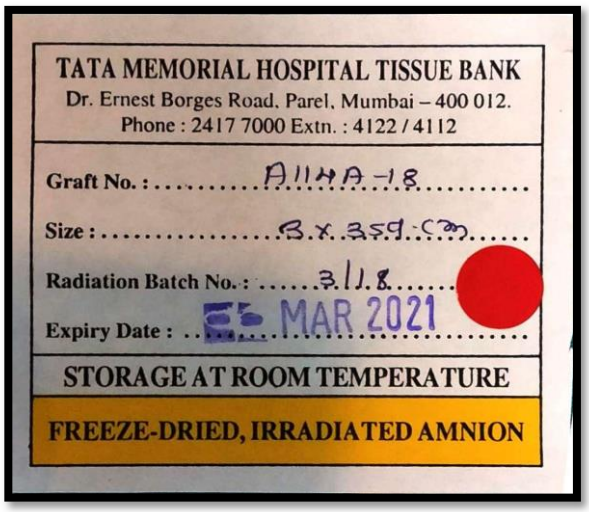


Fig 9: freeze- Dried, Irradiated Amnion

Fig 10: Amniotic Membrane Immediately After Placement in Saline

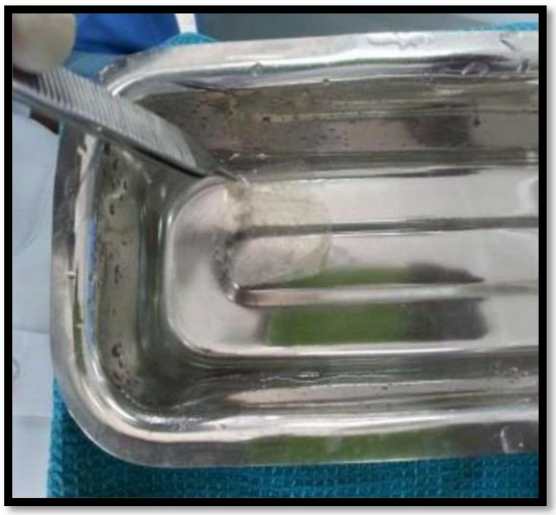


Fig 11: Amniotic Membrane After Placing in Saline Translucency Increases With Conversion To More Gel Form

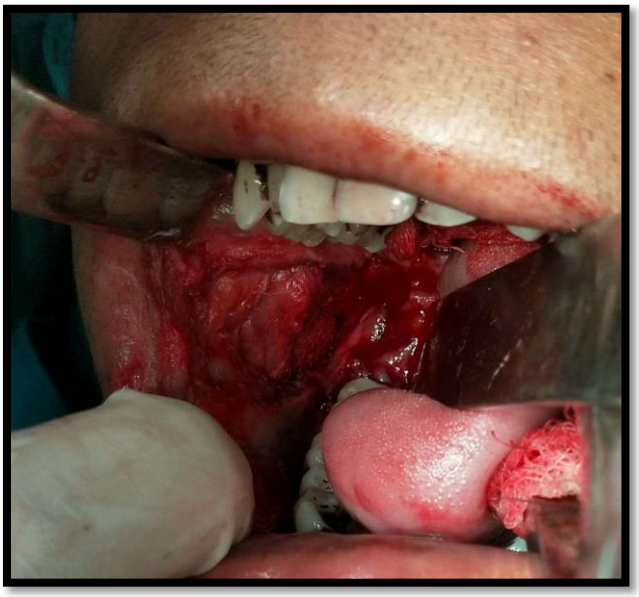


Fig 12: Placement of Amniotic Membrane

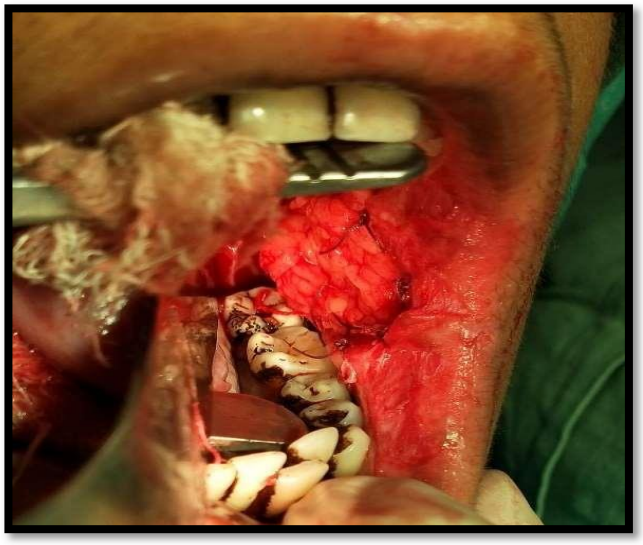


Fig 13: Harvesting and Reconstruction with BFP

POSTOPERATIVE DAY 7

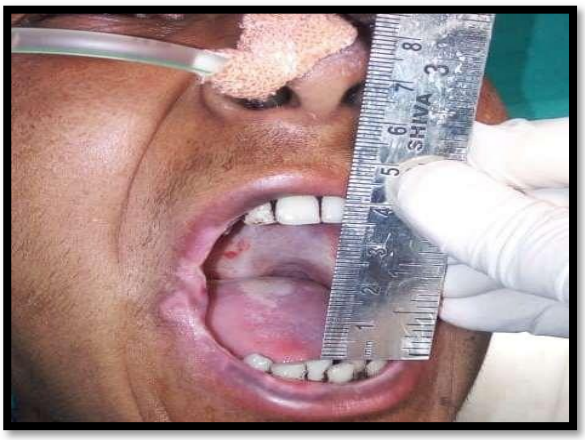


Fig 14: Mouth Opening – 35mm

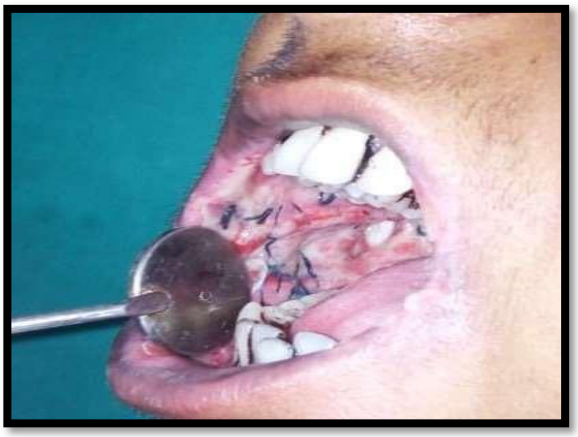


Fig 15: Right Buccal Mucosa (Amniotic Membrane)

Fig 16: Left Buccal Mucosa(BFP)

3 MONTH POST OPERATIVE

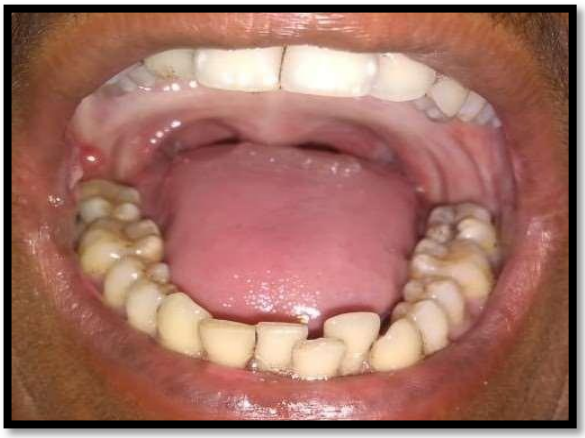


Fig 18: Position of Uvula

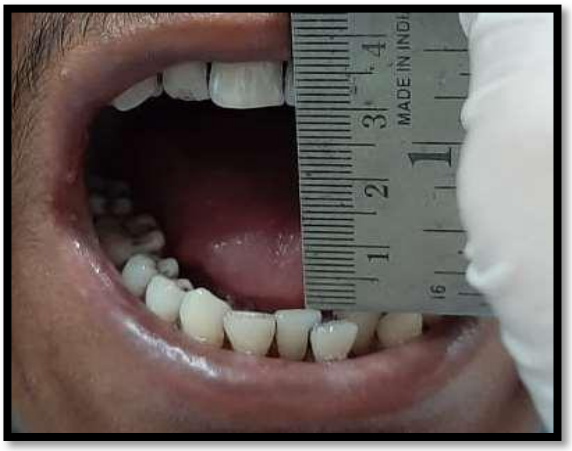


Fig 19: Mouth Opening 31mm

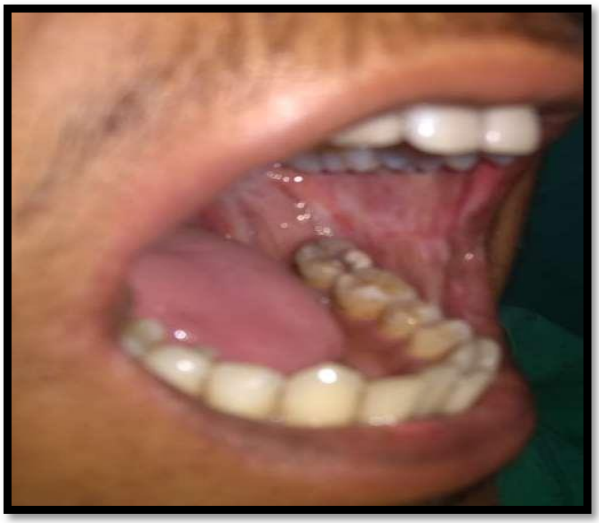


Fig 19: Left Buccal Mucosa

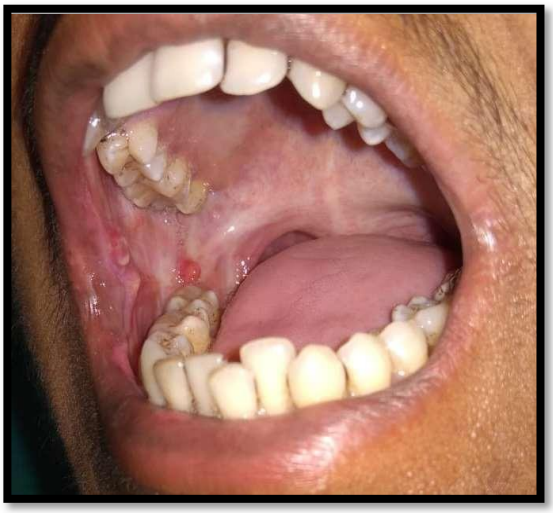


Fig 20: Right Buccal Mucosa

6 MONTH POST OPERATIVE

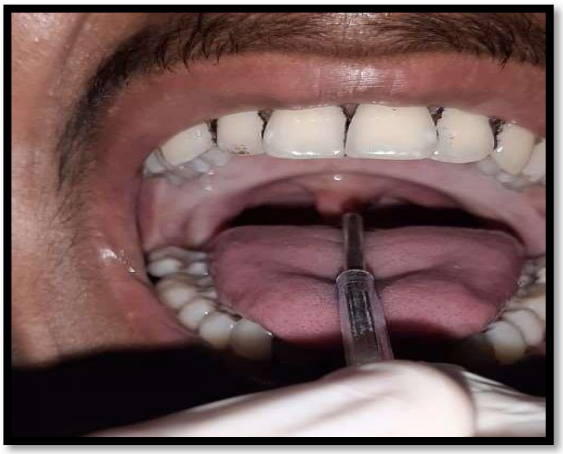


Fig 21: Position of Uvula

Fig 22: Mouth Opening – 33mm

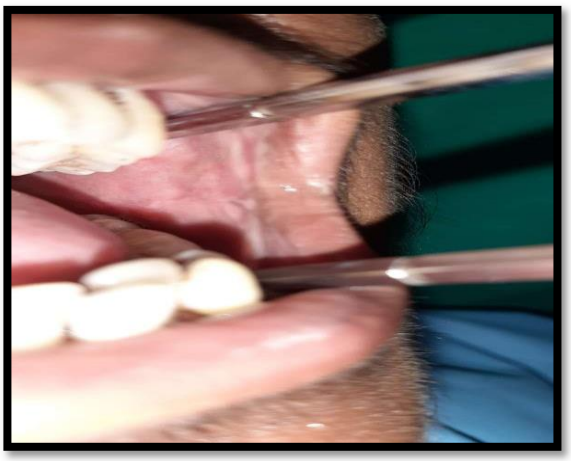


Fig 23: Left Buccal Mucosa

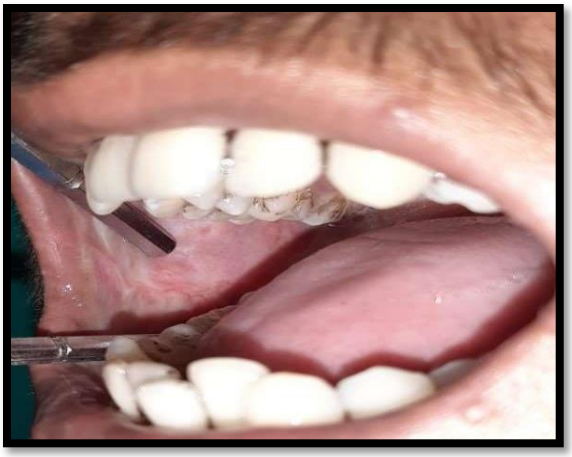


Fig 24: Right Buccal Mucosa

Radiographs

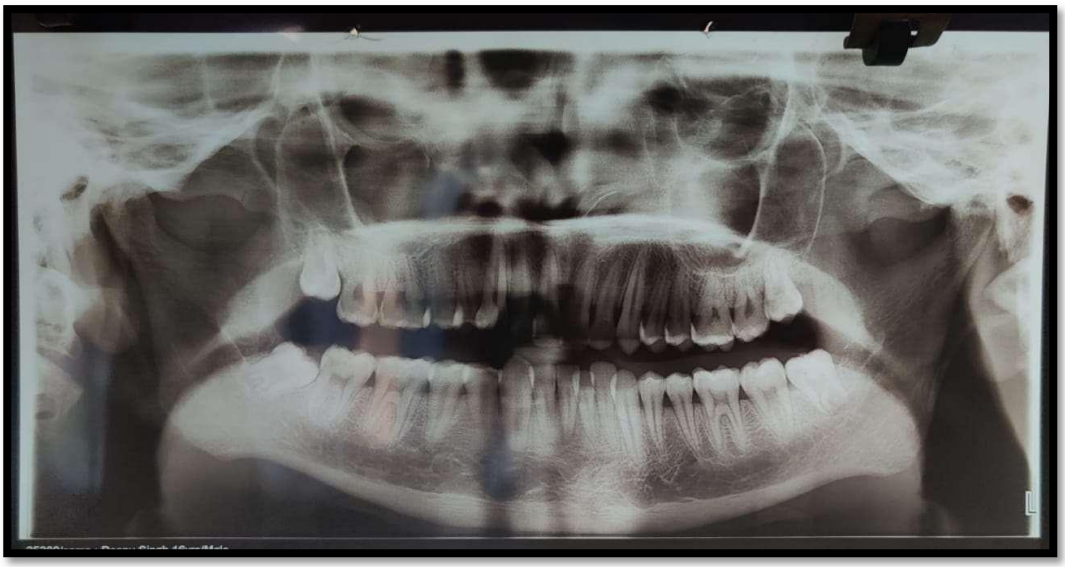


Fig 25: Pre-operative Radiograph

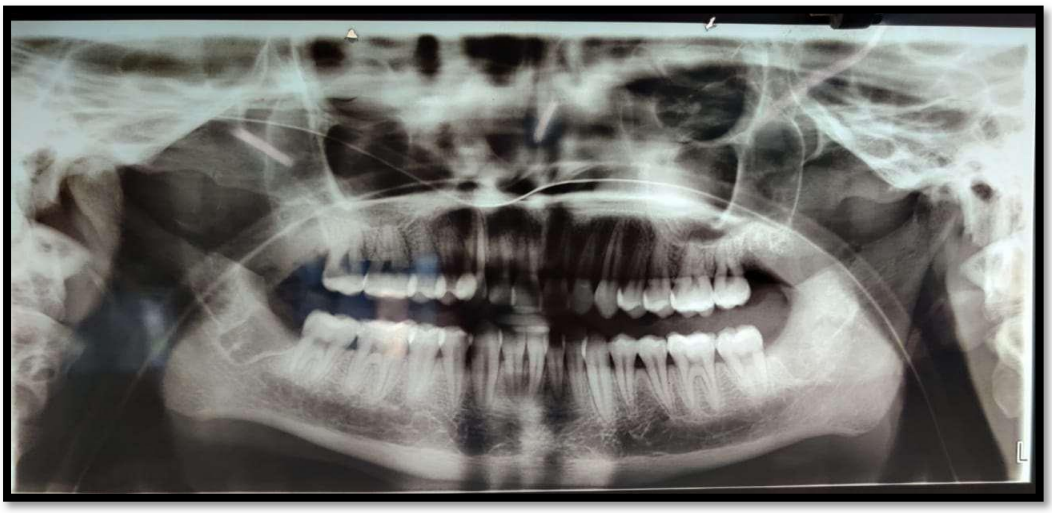


Fig 26: Post-operative Radiograph

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive data was reported for each variable. Descriptive statistics such as mean and standard deviation for continuous variables was calculated.

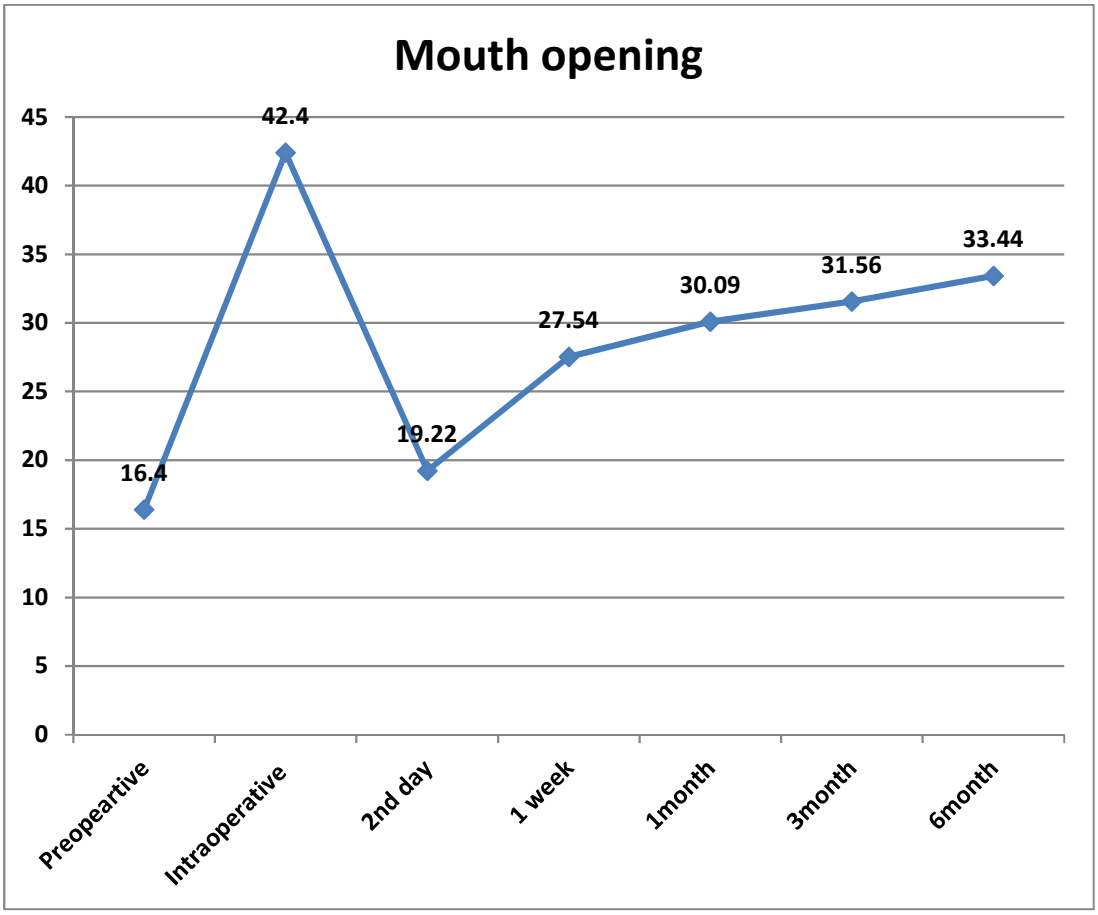
RESULTS

Summarized data was presented using Tables and Graphs. Shapiro Wilk test was used to check the normality of the data. As the data was found to be normally distributed bivariate analyses was performed using Paired t test. To compare proportions of a categorical outcome on right and left side Fisher's exact test is used. Level of statistical significance will be set at p-value less than 0.05

Table 1: Comparison of Mean mouth opening from baseline to different time intervals

	Mean	N	Std. Deviation	Std. Error Mean	P value
Preopeartive	16.40	5	5.128	2.293	
Intraoperative	42.40	5	4.159	1.860	0.003
2nd day	19.22	5	5.431	2.429	0.036
1 week	27.54	5	4.087	1.828	0.003
1month	30.09	5	4.393	1.965	0.009
3month	31.56	5	6.181	2.764	0.009
6month	33.44	5	8.927	3.992	0.032

Paired t test, level of significance set at $p<0.05$

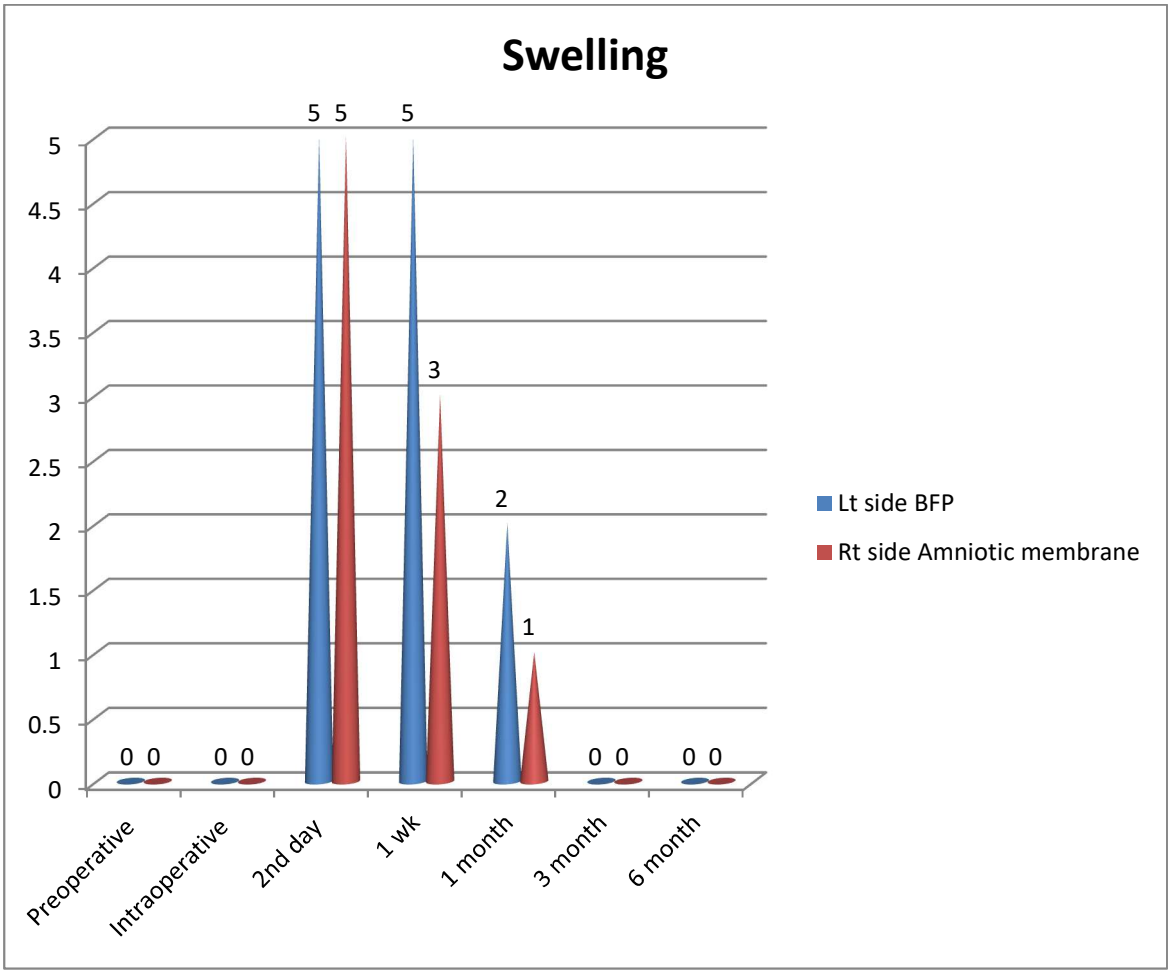


Graph 1 shows Comparison of Mean mouth opening from baseline to different time intervals. Significant differences were seen in mean mouth opening measurements preoperatively to intraopeartively, at 2nd day, 1 week, 1 month, 3 month and 6 months.

Table 2: Distribution of presence of swelling status at different time intervals

Swelling			Preoperative swelling	Intraoperative	2nd day	1 wk	1 month	3 month	6 month
Group	Lt side BFP	N	0	0	5	5	2	0	0
		%	0.00%	0.00%	100.0 %	100.0 %	40.0%	0.00%	0.00%
	Rt side Amniotic membrane	N	0	0	5	3	1	0	0
		%	0.00%	0.00%	100.0 %	60.0%	20.0%	0.00%	0.00%
Total		N	0	0	10	8	3	0	0
		%	0.00%	0.00%	100.0 %	80.0%	30.0%	0.00%	0.00%
P value			NA	NA	1.000	0.222	0.500	NA	NA

Fisher's exact test, level of significance set at $p<0.05$

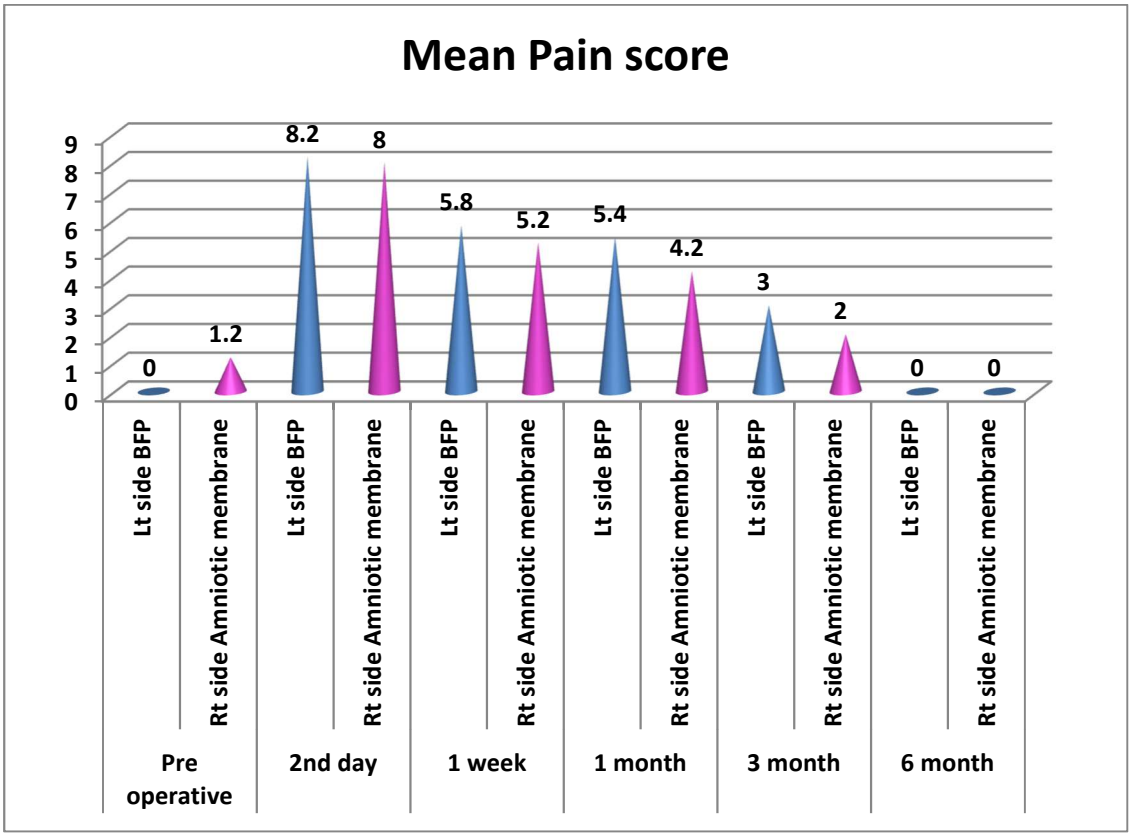


Graph 2 shows distribution of presence of swelling status at different time intervals. No significant difference was observed in the distribution of swelling status on left side using BFP and right side using amniotic membrane as $p>0.05$. Though at 1 week and 1 month swelling frequency was found to be more on side treated with BFP and at 6 months, on either side treated with BFP or amniotic fluid, swelling was not present.

Table 3: Mean Pain score comparison at different time intervals

Pain		Mean	N	Std. Deviation	Std. Error Mean	P value
pre operative	Lt side BFP	.00	5	.000	.000	.374
	Rt side Amniotic membrane	1.20	5	2.683	1.200	
2nd day	Lt side BFP	8.20	5	.837	.374	.621
	Rt side Amniotic membrane	8.00	5	1.414	.632	
At 1 week	Lt side BFP	5.80	5	.447	.200	.208
	Rt side Amniotic membrane	5.20	5	1.095	.490	
At 1 month	Lt side BFP	5.40	5	2.074	.927	.305
	Rt side Amniotic membrane	4.20	5	1.095	.490	
At 3 months	Lt side BFP	3.00	5	.707	.316	0.142
	Rt side Amniotic membrane	2.00	5	1.871	.837	
At 6 months	Lt side BFP	.00[a]	5	.000	.000	NA
	Rt side Amniotic membrane	.00[a]	5	.000	.000	

Paired t test, level of significance set at $p<0.05$

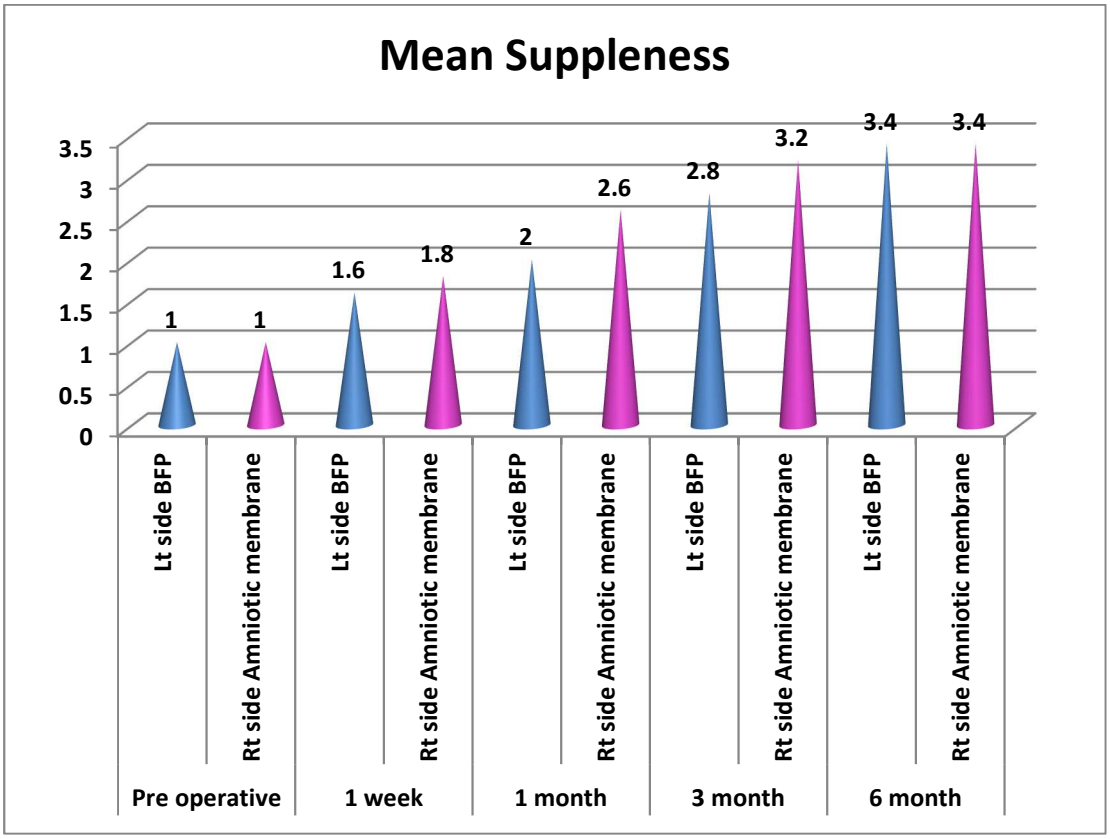


Graph 3 shows Mean Pain score comparison at different time intervals. No significant difference was seen in the mean pain score at different time intervals when sides were treated with either BFP and Amniotic membrane though pain score was found to be lesser among sides treated with amniotic membrane.

Table 4: Mean suppleness comparison at different time intervals

Suppleness		Mean	N	Std. Deviation	Std. Error Mean	P value
Pre	Lt side BFP	1.00[a]	5	.000	.000	NA
	Rt side Amniotic membrane	1.00[a]	5	.000	.000	
At 1 week	Lt side BFP	1.60	5	.548	.245	.374
	Rt side Amniotic membrane	1.80	5	.837	.374	
At 1 month	Lt side BFP	2.00	5	.707	.316	.208
	Rt side Amniotic membrane	2.60	5	.548	.245	
At 3 month	Lt side BFP	2.80	5	1.095	.490	.704
	Rt side Amniotic membrane	3.20	5	1.304	.583	
At 6 month	Lt side BFP	3.40	5	1.342	.600	1.000
	Rt side Amniotic membrane	3.40	5	1.342	.600	

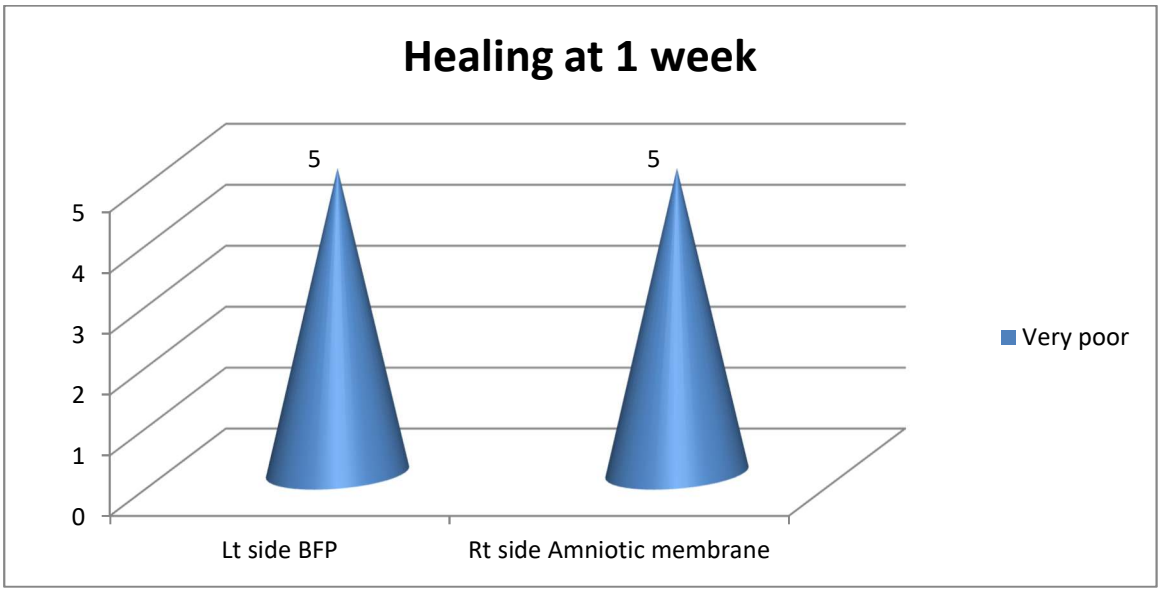
Paired t test, level of significance set at $p<0.05$



Graph 4 shows Mean Suppleness score comparison at different time intervals. No significant difference was seen in the mean suppleness score at different time intervals when sides were treated with either BFP and Amniotic membrane though suppleness score was found to be more among sides treated with amniotic membrane.

Table 5: Distribution of healing status on right and left side at 1 week

		1 week	Total
		Very poor	
Lt side BFP	Count	5	5
	%	100.0%	100.0%
Rt side Amniotic membrane	Count	5	5
	%	100.0%	100.0%
Total	Count	10	10
	%	100.0%	100.0%
P value	NA		

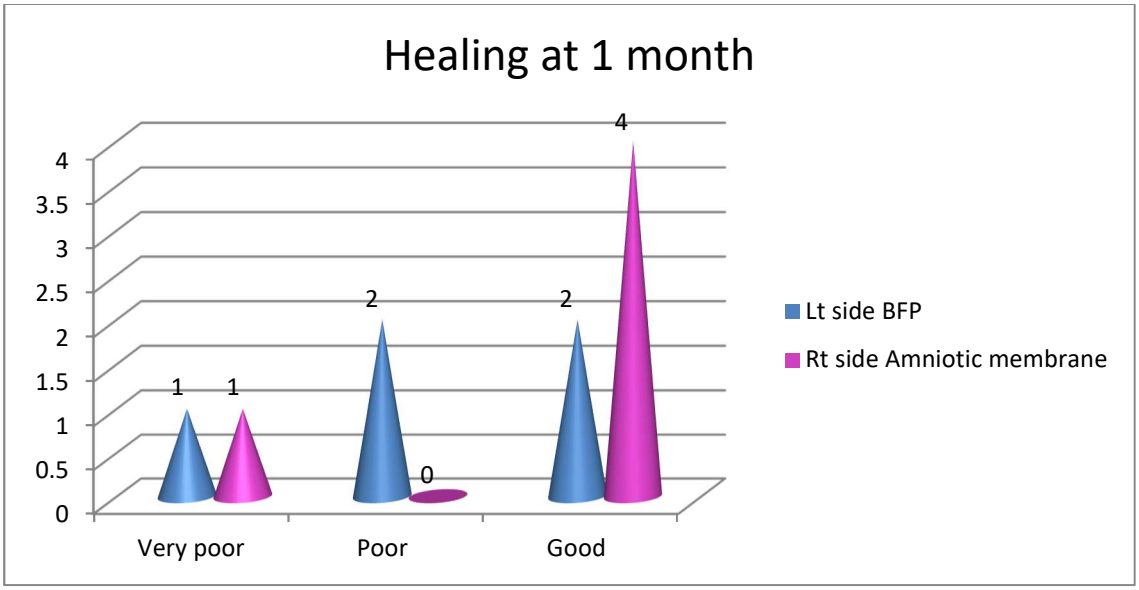


Graph 5 shows Distribution of healing status on right and left side at 1 week. At one week healing status was found be very poor among all sides treated with BFP or amniotic membrane.

Table 6: Distribution of healing status on right and left side at 1 month

		1 month			Total
		Very poor	Poor	Good	
Lt side BFP	Count	1	2	2	5
	%	20.0%	40.0%	40.0%	100.0%
Rt side Amniotic membrane	N	1	0	4	5
	%	20.0%	0.0%	80.0%	100.0%
Total	N	2	2	6	10
	%	20.0%	20.0%	60.0%	100.0%
P value	0.264				

Fisher's exact test, level of significance set at $p<0.05$

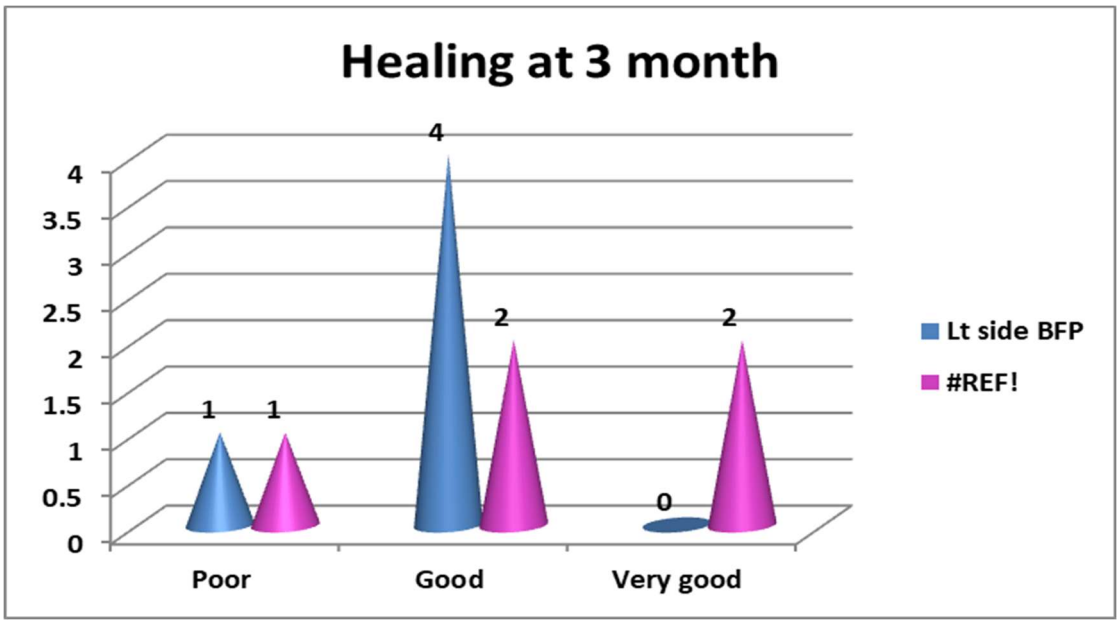


Graph 6 shows Distribution of healing status on right and left side at 1 month. No significant difference was seen in the distribution status of healing status when treated with BFP or amniotic membrane. Though healing status was found to be good (80%) among sides treated with amniotic fluid as compared to BFP (40%).

Table 7: Distribution of healing status on right and left side at 3 month

		At 3month			Total
		Poor	Good	Very good	
Lt side BFP	N	1	4	0	5
	%	20.0%	80.0%	0.0%	100.0%
Rt side Amniotic membrane	N	1	2	2	5
	%	20.0%	40.0%	40.0%	100.0%
Total	N	2	6	2	10
	%	20.0%	60.0%	20.0%	100.0%
P value	0.264				

Fisher's exact test, level of significance set at $p<0.05$

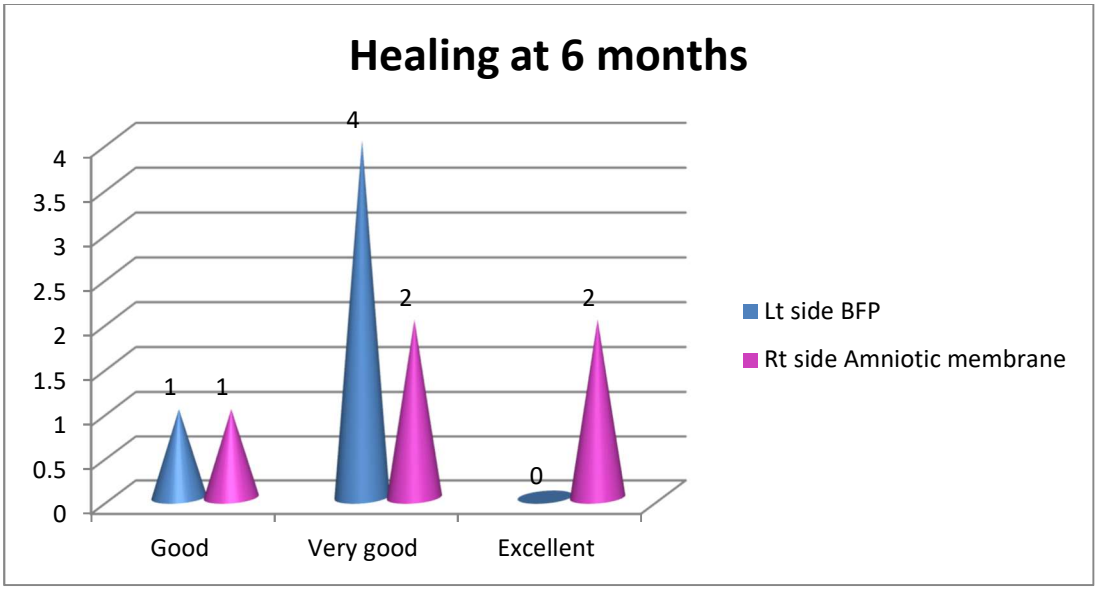


Graph 7 shows Distribution of healing status on right and left side at 3 month. No significant difference was seen in the distribution status of healing status when treated with BFP or amniotic membrane. Though healing status was found to be very good (40%) among sides treated with amniotic fluid as compared to BFP (0.0%).

Table 8: Distribution of healing status on right and left side at 6 month

		At 6month			Total
		Good	Very good	Excellent	
Lt side BFP	N	1	4	0	5
	%	20.0%	80.0%	0.0%	100.0%
Rt side Amniotic membrane	N	1	2	2	5
	%	20.0%	40.0%	40.0%	100.0%
Total	N	2	6	2	10
	%	20.0%	60.0%	20.0%	100.0%
P value	0.264				

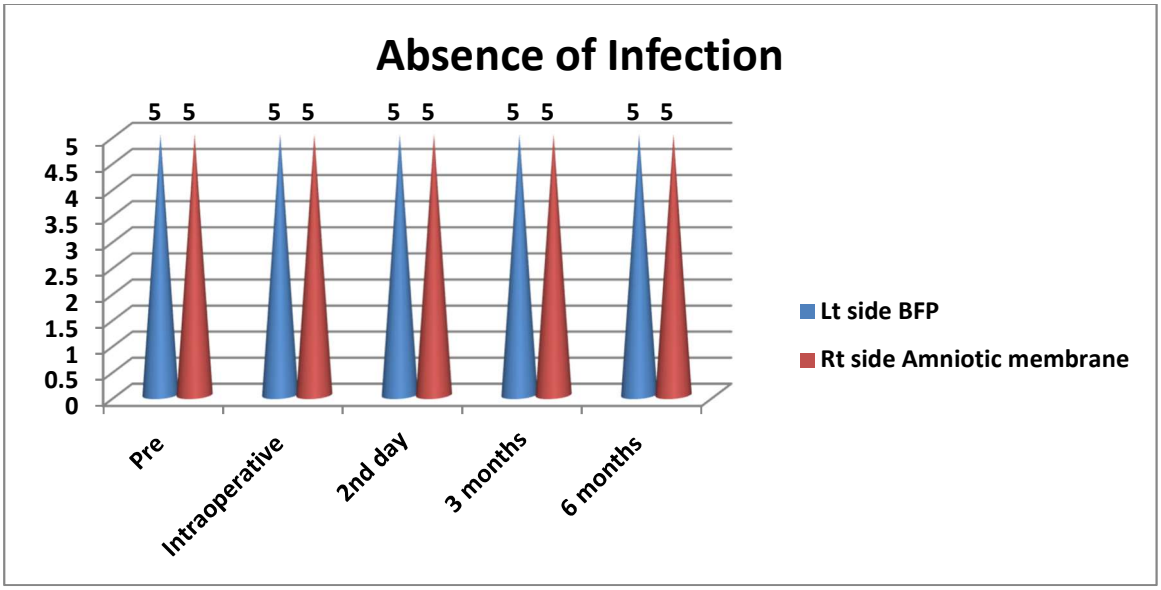
Fisher's exact test, level of significance set at $p<0.05$



Graph 8 shows Distribution of healing status on right and left side at 6 month. No significant difference was seen in the distribution status of healing status when treated with BFP or amniotic membrane. Though healing status was found to be excellent (40%) among sides treated with amniotic fluid as compared to BFP (0.00%).

Table 9: Distribution of infection status at different time intervals

		Pre	Intraoperative	2nd day	3 months	6 months	Total
		Absent	Absent	Absent	Absent	Absent	
Lt side BFP	N	5	5	5	5	5	5
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Rt side Amniotic membrane	N	5	5	5	5	5	5
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Total	N	10	10	10	10	10	10
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
P value	NA						

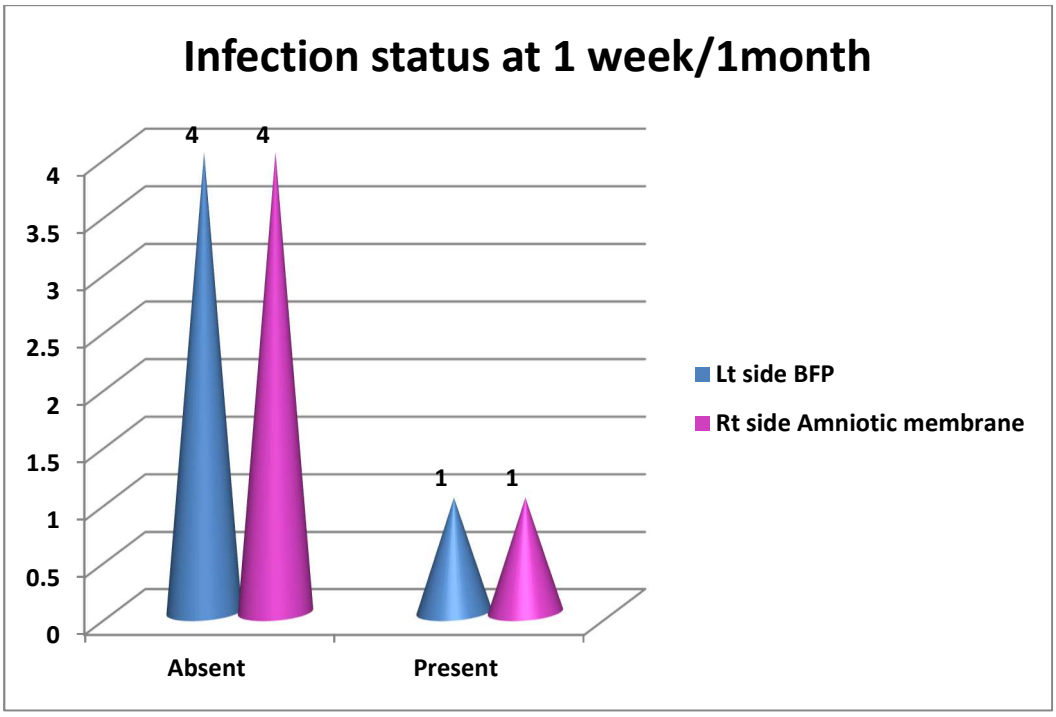


Graph 9 shows Distribution of infection status at different time intervals. Infection was not found among sides treated with BFP and amniotic membrane preoperatively, intraopeartively, on 2nd day, 3 months and 6 months.

Table 10 : Distribution of infection status at 1 week

		At 1wk/ 1 month		Total
		Absent	Present	
Lt side BFP	N	4	1	5
	%	80.0%	20.0%	100.0%
Rt side Amniotic membrane	N	4	1	5
	%	80.0%	20.0%	100.0%
Total	N	8	2	10
	%	80.0%	20.0%	100.0%
P value	0.778 NS			

Fisher's exact test, level of significance set at $p<0.05$

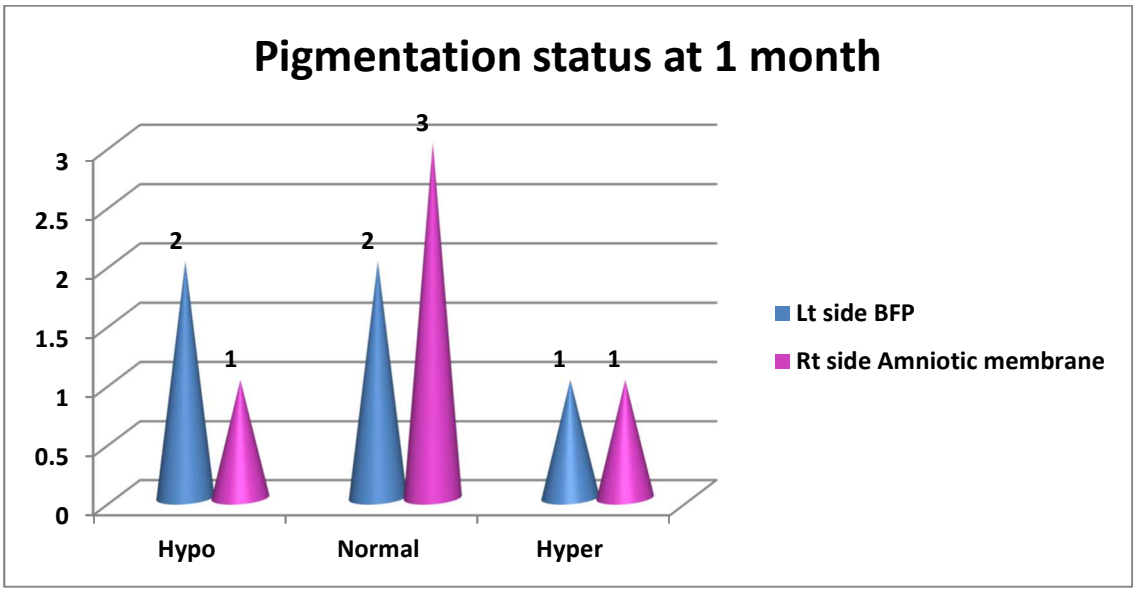


Graph 10 No significant difference was seen in the distribution of infection status at 1 week and 1 month among sides treated with BFP and amniotic membrane as $p>0.05$

Table 11 : Distribution acc to pigmentation status at 1 month

		At 1month			Total
		Hypo	Normal	Hyper	
Lt side BFP	N	2	2	1	5
	%	40.0%	40.0%	20.0%	100.0%
Rt side Amniotic membrane	N	1	3	1	5
	%	20.0%	60.0%	20.0%	100.0%
Total	N	3	5	2	10
	%	30.0%	50.0%	20.0%	100.0%
P value	0.766				

Fisher's exact test, level of significance set at $p<0.05$

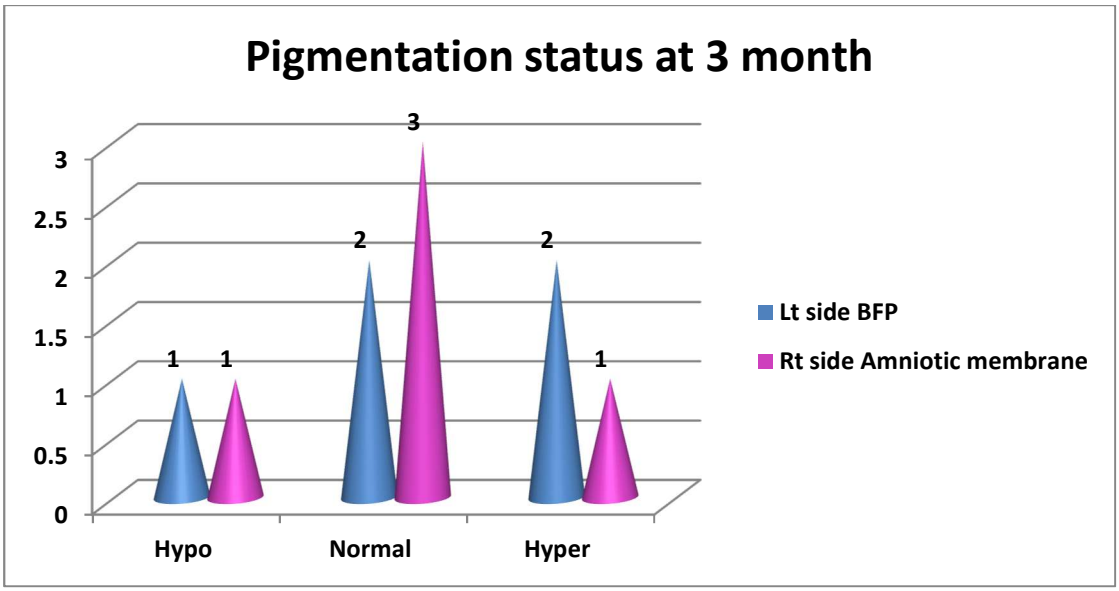


Graph 11 shows Distribution acc to pigmentation status at 1 month. No significant difference was seen in the distribution acc to pigmentation status as 1 month among sides treated with BFP and Amniotic membrane. Though normal pigmentation was seen more among sides treated with Amniotic membrane.

Table 11: Distribution acc to pigmentation status at 3 month

		3 month			Total
		Hypo	Normal	Hyper	
Lt side BFP	N	1	2	2	5
	%	20.0%	40.0%	40.0%	100.0%
Rt side Amniotic membrane	N	1	3	1	5
	%	20.0%	60.0%	20.0%	100.0%
Total	N	2	5	3	10
	%	20.0%	50.0%	30.0%	100.0%
P value	0.766				

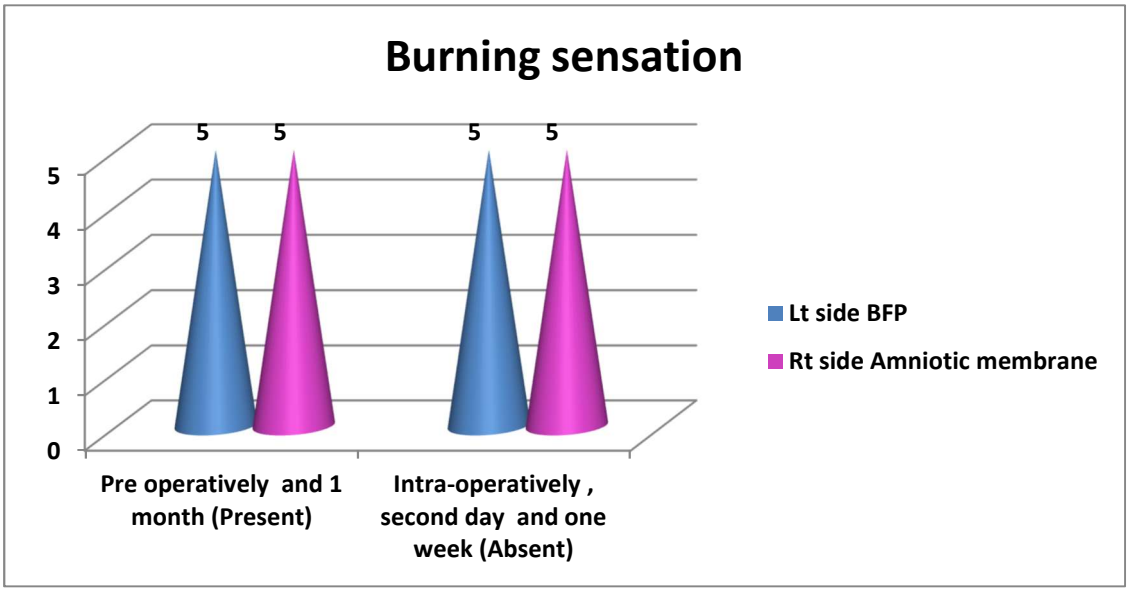
Fisher's exact test, level of significance set at $p<0.05$



Graph 12 shows Distribution acc to pigmentation status at 3 month. No significant difference was seen in the distribution acc to pigmentation status as 3 month among sides treated with BFP and Amniotic membrane. Though normal pigmentation was seen more among sides treated with Amniotic membrane.

Table 13: Distribution acc to burning status preoperatively, intraoperatively, second day, one week and one month

		Pre operatively and 1 month	Intra-operatively , second day and one week
		Present	**Absent**
Lt side BFP	N	5	5
	%	100.0%	100.0%
Rt side Amniotic membrane	N	5	5
	%	100.0%	100.0%
Total	N	10	10
	%	100.0%	100.0%
P value		NA	NA

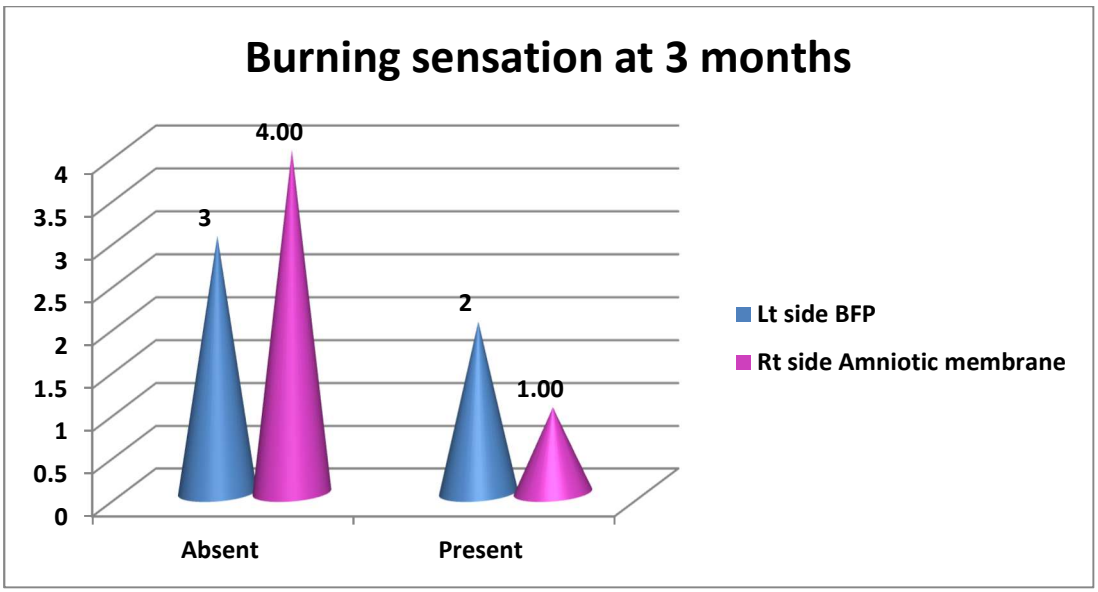


Graph 13 shows Distribution acc to burning status preoperatively, intraoperatively, second day, one week and one month. Burning sensation was present among all the subjects preoperatively and at 1 month where as it was absent in all the subjects, Intra-operatively, on second day and one week.

Table 14: Distribution acc to burning status at 3 months

		At 3 months		Total
		Absent	Present	
Lt side BFP	N	3	2	5
	%	60.0%	40.0%	100.0%
Rt side Amniotic membrane	N	4	1	5
	%	80.0%	20.0%	100.0%
Total	N	7	3	10
	%	70.0%	30.0%	100.0%
P value	0.490			

Fisher's exact test, level of significance set at $p<0.05$

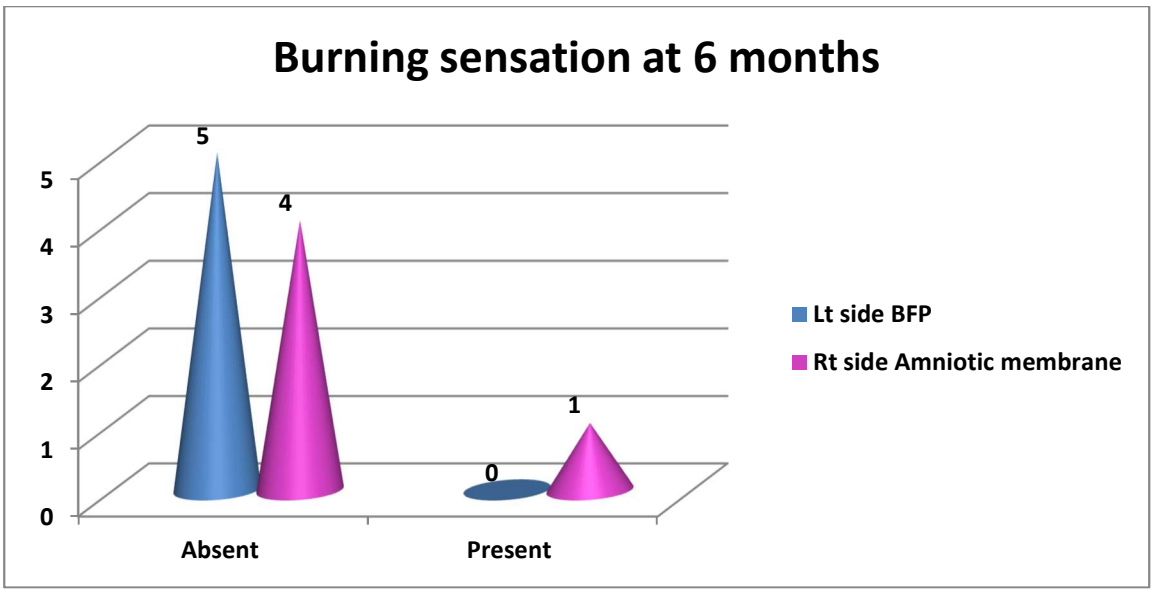


Graph 14 shows Distribution acc to burning status at 3 months. No significant difference was seen in the burning status on right and left side at 3 months.

Table 15: Distribution acc to burning status at 6 months

		At 6 months		Total
		Absent	Present	
Lt side BFP	N	5	0	5
	%	100.0%	0.0%	100.0%
Rt side Amniotic membrane	N	4	1	5
	%	80.0%	20.0%	100.0%
Total	N	9	1	10
	%	90.0%	10.0%	100.0%
P value	0.292			

Fisher's exact test, level of significance set at $p<0.05$



Graph 15 shows Distribution according to burning status at 6 months. No significant difference was seen in the burning status on right and left side at 6 months.

DISCUSSION

OSMF is an insidious, chronic disease affecting any part of the mouth & sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it's always associated with juxtaepithelial inflammatory reaction, followed by a fibroelastic change of the lamina propria with epithelial atrophy, resulting in stiffness of the oral mucosa and causing trismus and inability to eat. (Pindborg&Sirsat 1966).[1]

OSMF has various sorts of etiological factors, among which tobacco and chewing areca nut is the common and accepted one. In our study, all patients had a positive history of chewing some sort of tobacco or areca nut or combination of both for variable duration. Epidemiological data and intervention studies suggest that arecanut is the main aetiological factor of OSMF (Pindborg et al[1], Murti et al[92]). Other etiological factors suggested are chillies, lime, tobacco, nutritional deficiencies such as iron and zinc, immunological disorders, and collagen disorders.

The diagnostic criteria for OSMF in early stages having clinical features like burning sensation of mucosa, intolerance to hot and spicy food, mucosal blanching, vesiculation, excessive salivation, pigmentation change, ulceration, altered taste sensation, dryness of mouth, and recurrent stomatitis . Fibrosis is commonest followed by stiffness within buccal mucosa, taste bud and faucial pillars. Fibrotic bands become more palpable which run vertically in the cheek region and circumferentially within the lips. Progressively, this leads to inability in opening the mouth. In advanced cases, shrunken and bud like uvula, restricted function of soft palate and limited tongue movements are seen.[93,94]

The mainstay within the treatment of OSMF is targeting on attempts to enhance the mouth opening and relieve the symptoms by medicinal or surgical means. Gradual restriction of mouth opening has health related issues and social consequences.

Many conservative treatment modalities for OSMF has been proposed. Which include oral treatment with use of vitamins, antioxidants and Iron supplements, Zinc & topical application of Gold,[95] Iodides & various intralesional injections were used, i.e Hyaluronidase, Hydrocortisone,[96] Placental extract, Triamcinolone, Interferon gamma, and enzymes such as Collagenase, Chymotrypsin. The medical management has been extensively reported within the review literature by Kerr et al.[97]

The surgery is the method of choice in patients with marked limitation of mouth opening . The subsequent surgical modalities are used to release fibrous bands and covering of the raw areas with split thickness skin grafting, bilateral nasolabial flaps, palatal island flaps, tongue flaps, temporalis myotomy, and coronoidectomy.

Surgical therapy is beneficial in cases with severe trismus and in those patients who were not responding to the conservative treatment. With surgical therapy, oral mucosa regains and retain its normalcy. There is reduced risk of oral cancer.

Surgical wounds of the oral mucosa (like other Wound) heal by granulation and then epithelialization. In General Surgery it is well known fact that grafted wounds heal more faster than open wounds & their incidence of infection and degree of scar formation gets reduced, when wounds are fully covered with biological materials instead of those unadequately covered or dressed using non-biological materials. So, Open wound surfaces should be covered with appropriate graft materials, as lost structure should get replaced with its equivalent according to surgical principles.[68]

Although improvement in reconstruction methods & materials is seen in recent past, substitution of lost structure in oral cavity has been still a challenge in comparision to other organs like skin. Due to issue arising with tissue mobility, regular movements of the cheek and tongue by mastication, articulation and deglutition. The moist environment provided by saliva & contamination caused due to organism present in food which may be compounded by poor oral hygiene may interfere with graft adherence and retention leading to inadequate healing of wound, epithelialization of wound and significant scar contraction. The oral functions can be restricted with any grade of residual scarring. For this issue, biological dressings been in use because they can remain stable for an adequate time period, may be an important factor for faster and adequate wound healing.[57] Various grafts or wound dressing materials are tried in oral and maxillofacial surgery only autogenous skin graft and xenogenous collagen products are primarily used.[98] Autogenous grafts are considered as ideal graft materials immunologically.[58,99] They efficiently replace the lost structure & provide good surface coverage. But with limitations like limited availability, donor site morbidity, difficult techniques in harvesting and Skin grafts when used will always have the color mismatch to the grafted area.

In oral surgery, collagen based bio-dressings, mostly bovine collagen sheets, are extensively used. The high percentage of equivalence of amino acid sequences between humans and bovine collagen (98% in α1 and 93% in α 2 chain),[100] conformability to the mucosa, bioactivity and a hemostatic effect make it an excellent choice as a biological dressing.[101,102], All collagen membranes gradually undergo collagenolysis which are compounded by the oral environment and get sloughed off at

last. Even though crosslinking alleviates this weakness, helps in reducing or suppressing antigenicity but changes its original properties of the collagen.

Non acceptance and inflammation of the grafted area, high cost, loss of properties with processing, and limited simple access of those allogeneic or xenogenous membranes mandate an urgent got to look.[69]

In the present study, The Human Amniotic Membrane and buccal fat pad were used as a biological dressing to manage post-surgical oral submucous fibrosis and therefore the effectiveness and equivalence of these novel graft material as a replacement to be use in oral & maxillofacial surgery were assessed.

HUMAN AMNIOTIC MEMBRANE (AM) is the deepest semiopaque layer of the placenta that possesses several unique characteristics. There are 3 layers of AM: an epithelial monolayer, a basement membrane, and an underlying stroma. Nutrients are supplied directly by diffusion, from the amniotic fluid and/or from the underlining decidua. The amniotic epithelial cell layer is a single layer of flat, cuboidal and columnar cells that are in direct contact with the amniotic fluid. Normal AM having thickness of 0.02-0.5 mm, which is equal to 6-8 cells. Physical properties of AM, such as thinness and tensile strength provided by the basement membrane, warrant it to function as an anatomic barrier to fibrous tissue proliferation.[104] In amniotic basement membrane collagen type I, III, IV, V, and VII, laminin and fibronectin have been identified,[105,106] and when the amniotic membrane is applied to the surgical site, stromal matrix provided a biological rather than just a mechanical relationship.[107] Properties of AM such as Anti-adhesiveness; non immunogenicity; antimicrobial action; anti -inflammatory and analgesic properties are well documented in literature. It also stimulates secondary epithelialization; protects the wound; vascularize healthy

granulation tissue and promotes angiogenesis in adjacent tissues.[44,108,109,110] It is easily available, inexpensive and can be used fresh or lyophilized and can be stored at room temperature after sterilization by gamma irradiation. It are often easily procured, processed and transported.

In 1910, Davis,[4] first showed the placental membranes uses for skin transplantation. In 1913 Stern and Sabella respectively used amniotic membrane in treatment of skin burns and superficial wounds.[111] Since then hundreds of studies proposed on application of AM in almost all the fields of medicine including plastic surgery, abdominal adhesions, ophthalmology, burn care, tissue engineering, oncology, etc. Hence proving to be an excellent biological dressing with equivalent qualities of an ideal dressing. Amniotic Materials have shown to improve, Soft tissue repair , Skin burn healing , General wound covering , Acute Chemical Burns of the Eye , Osteogenesis , Chronic, Ulcer repair, Conjunctival Defect repair, Cartilage regeneration , Skin Grafting , Angiogenesis or Tendon repair. Many studies has provided evidence that cells which are derived from the amniotic membrane are able to differentiate into many other kinds of mature cells, including adipocytes, osteocytes, chondrocytes, myocytes and hepatocytes. Above observations suggested that HAM contains stem cell-like cells properties and could be used as an alternative source of cells for regenerative medicine.[112]

For HAM preparation various techniques such as heat-drying, air-drying, lyophilization, treatment with cold glycerol, and cryopreservation methods are in use . Amniotic membranes, described by Atiyeh et al,[109] help in preserving a healthy excised wound bed; maintain a low bacterial count in infected wounds; prevent the loss of protein, electrolytes, fluids, and energy; reduce the risk of contamination;

avoid bulky dressings; minimise pain; and accelerate epithelial regeneration, so reducing the duration of hospital stay.[9,113]

The use of the BFP for reconstruction in oral defects was studied to evaluate its feasibility as a flap for the reconstruction of oral defects, comparison with the buccal flap. It was found that harvesting of the BFP did not produce any marked defect in the cheek.

In reference to the oral cavity, buccal fat pad harvesting is a technically easy procedure, both donor and recipient sites are contiguous in the oral cavity, there is no visible scar in the donor area, the matter of losing transplanted adipose tissue within the long-term may be negligible factor because the anatomic proximity of the donor and recipient sites permits rapid grafting without having the fatty graft too long outside the body of the patient.[114] The BFP is not totally free of complication. It can cause severe atrophy in chronic cases and the anterior reach is something inadequate, leaving a raw area which heals by secondary intension and subsequently leading to relapse according to *Tideman et al* .[115]

Borle and Borle[116] reported disappointing results with split skin grafts in covering the mucosal defect after excision of fibrotic bands in the management of oral submucous fibrosis. Khanna and Andrade[73] reported the incidence of shrinkage, contraction, and rejection of split skin grafts to be very high, leading to poor oral conditions and recurrence in 12 cases of oral submucous fibrosis. Hao[50] reported one (5%) failure and one (5%) complication in 21 patients after applying a pedicled buccal fat pad flap in the reconstruction of oral defects. Similar results were reported by Dean et al.[113]; only one (3%) out of 32 patients showed partial loss of the buccal fat pad flap. This suggests that as compared to the split skin graft, AlloDerm, and buccal fat pad flap,

the HAM graft is a better option for oral submucous due to low chances of infection and graft failure.

This study suggested that in comparison between split skin graft, and buccal fat pad flap, the HAM graft is an ideal option for oral submucous fibrosis due to low chances of infection and graft failure. Human amniotic membrane is used in various form such as dried, frozen, irradiated and lypholized form. In the present study we have evaluated the versatility of Deep Freeze-Dried HAM in OSMF patients in oral and maxillofacial surgery.

Present study was conducted with an aim to achieve the outcome in terms of mouth opening and reduction in various symptoms. This prospective study included 5 patients diagnosed with OSMF and they were divided into 2 surgical sites left and right buccal mucosa.Group I(left buccal mucosa) comprised with grafting of the defect with BFP and in group II(right buccal mucosa) using freeze dried amniotic membrane as wound dressing material .

The results observed are discussed as follows:

Maximum mouth opening

Comparison of Mean mouth opening from baseline to different time intervals. Significant differences were seen in mean mouth opening measurements preoperatively to intraopeartively, at 2nd day, at 1st week, at 1 month interval, at 3 month and 6 months interval.

Choi and Tseng[117] stated that amniotic membrane inhibits the expression of TGF-β receptors in fibroblasts, resulting in less fibrosis.

Swelling

No significant difference was observed in the distribution of swelling status on left side using BFP and right side using amniotic membrane as $p>0.05$. Though at 1 week and 1 month swelling frequency was found to be more on side treated with BFP and at 6 months, either side treated with BFP or amniotic membrane, swelling was not present.

Marsh et al[118] explained in their study that As an emerging anti-inflammatory and anti-fibrotic treatment, the use of human allograft membrane has proven to be both safe and effective in humans thus far and continues to pique interest as an alternative therapy option. The anti-inflammatory properties of amniotic membrane were exemplified, as there was no evidence of inflammation notable in their study.

Pain:

No significant difference was seen in the mean pain score at different time intervals when sides were treated with either BFP and Amniotic membrane though pain score was found to be lesser among sides treated with amniotic membrane.

Hajiiski and Anatassov[119]described HAM as a biological dressing agent, which resulted in significant reducing pain due to adhesion to the wound surface and dermal nerve endings. It also prevents drying of wound surface, which help in fast healing of wound. The adherence of AM is either due to the result of fibrin collagen interaction or may be the result of fibrovascular ingrowth into collagenous stroma as seen as a fibrin like whitish substance seen beneath the smooth wound surfaces in almost all the patients upon removal of the pressure dressing[1,6].

Suppleness

No significant difference was seen in the mean suppleness score at different time intervals when sides were treated with either BFP and Amniotic membrane though suppleness score was found to be more among sides treated with amniotic membrane.

Kothari et al in[69] also concluded that grafts of amniotic membrane are viable and reliable for covering of the raw surface, prevent secondary contraction and maintain postoperative depth.

Healing

Healing status on right and left side at 1 month. No significant difference was seen in the distribution status of healing status when treated with BFP or amniotic membrane. Though healing status was found to be good (80%) among sides treated with amniotic membrane as compared to BFP (40%).

Healing status on right and left side at 3 month. No significant difference was seen in the distribution status of healing status when treated with BFP or amniotic membrane. Though healing status was found to be very good (40%) among sides treated with amniotic membrane as compared to BFP (0.0%).

Healing status on right and left side at 6 month. No significant difference was seen in the distribution status of healing status when treated with BFP or amniotic membrane. Though healing status was found to be excellent (40%) among sides treated with amniotic membrane as compared to BFP (0.00%).

Samandari et al[5,63] this study proposed that after the 1st week, when wound area was seen after removal of dressing, a white necrotic slough was seen over the graft area. Similarly at the end of the 2nd week, a slight hyperemic mucosal tissue was noticed

and a fully epithelialized wound was seen after 1 month. By the third month, the graft area was not distinguishable from the normal mucosa.

Infection

Infection was not found among sides treated with BFP and amniotic membrane preoperatively, intraopeartively, on 2nd day, 3 months and 6 months.

No significant difference was seen in the distribution of infection status at 1 week and 1 month among sides treated with BFP and amniotic membrane as $p>0.05$

Several studies stated that Amniotic membrane is equalvalent to isograft & superior to allograft due to reduction in chances of bacterial load. Similarly Qureshi IZ et al[105] and Baradaran-rafii A et al[108]explained in their study that Amniotic membrane has protein called cystatin E, the analogue of cysteine proteinase inhibitor, which showed antiviral properties. In clean surgical wounds, the hemostatic property of amniotic stromal layer due to presence of collagen fibers prevents hematoma formation thereby reducing microbial accumulation and hence the risk of infection.

Donald E et al[120] study proposed on Use of Dehydrated Amniotic Membrane in Wound Management. Showed results that the amniotic membrane allograft will incorporate early into the wound bed within 1 week to 2 weeks.

Pigmentation

Pigmentation status at 1 month. No significant difference was seen in the distribution acc to pigmentation status as 1 month among sides treated with BFP and Amniotic membrane. Though normal pigmentation was seen more among sides treated with Amniotic membrane.

Pigmentation status at 3 month. No significant difference was seen in the distribution acc to pigmentation status as 3 month among sides treated with BFP and Amniotic membrane. Though normal pigmentation was seen more among sides treated with Amniotic membrane.

Sikder et al[121] and Ehtaih sham and sultana[122] used amniotic membrane for the reconstruction of buccal mucosal defects. Graft was restored successfully without any complications. They found good reconstruction, postoperative function, and good esthetics.

Burning sensation

Burning status preoperatively, intraoperatively, second day, one week and one month. Burning sensation was present among all the subjects preoperatively and at 1 month where as it was absent in all the subjects, Intra-operatively , on second day and one week.

Burning status at 3 months. No significant difference was seen in the burning status on right and left side at 3 months.

Burning status at 6 months. No significant difference was seen in the burning status on right and left side at 6 months.

CONCLUSION

The specific purpose of this study was to evaluate the efficacy of freeze dried irradiated amniotic membrane v/s BFP as grafting and following conclusion can be drawn out of the study.

- As compared to BFP flap the amniotic membrane resilience encourages immediate commencement of mouth opening exercises resulting in improved mouth opening. Spontaneous epithelization makes the BFP more rational, reliable and convenient surgical technique.
- As compared to BFP the amniotic membrane serves as a satisfactory substitute, because it provided excellent function, offers ease of surgery and had little postoperative morbidity and good patient acceptance
- As compared to BFP the amniotic membrane was easy to use and shown to have good hemostatic property and easily used as grafting material in the surgical management of OSMF having good functional and esthetic outcome taking into account the improved suppleness after epitheliazation.
- None of the patients had shown any allergic reaction Post-operatively
- Even though the oedema was found to be stastically significant postoperatively in BFP As compared to the amniotic.
- It was interesting to observe that although grafting with the amniotic membrane trismus band resection and extraction of all 3rd molar coupled with coronoidectomy yields better long term results As compared to BFP. This leads us to believe that probably 3rd molar removal plus coronoidectomy are the best determinants for

stability of postoperative mouth opening, amniotic membrane in addition adds to suppleness of mucosa.

- In our study, we discovered that human amniotic membrane found to be a potential grafting material for oral cavity reconstruction surgeries with useful inherent properties and promising results in the repair of post-surgical oral mucosal defects. The results showed that the membrane was useful for all patients examined during this study.
- This study demonstrated that using amniotic membrane could accelerate healing process especially in its early stage. It reduces the overall inflammatory phase of healing and remarkably enhances re-epithelialization of oral wound surface.
- The amniotic membrane was found easy to handle and easy to use with inherent hemostatic property which is observed in all patients.
- No patients had shown any evidence of any complications.
- Infection chances was negligible as shown in patients.
- There was fair to good pain control observed in patients throughout there postoperative period. Fast Epithelialization with good suppleness of tissues and with reduced restriction of mouth opening.
- Although the number of cases was small, outcome indicated that the human AM is biologically ideal graft for oral wounds and could be used as clinical alternative for various repair surgery for oral defects.
- A further study with more sample size and with increased period of observation would prove to be more valuable for evaluation of HAM effectiveness.

- Mostly patient returned to normal diet within 1 month & there was fair to good pain control throughout the postoperative period.

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Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIIth Institutional Ethics Sub-Committee

IEC Code: 34 **BBDCODS/01/2019**

Title of the Project: Comparison of the Efficacy of Amniotic Membrane V/S Buccal Fat Pad in Treatment of Oral Submucous Fibrosis.

Principal Investigator: Dr. Shipra Sharma **Department:** Oral & Maxillofacial Surgery

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Shipra Sharma,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 10th January 2019.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics & Dentofacial Orthopedics, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Lakshmi Bala
31/01/19
Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Forwarded by:

(Dr. Rajkumar)
Principal
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BBDCODS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Comparison of the Efficacy of Amniotic Membrane V/S Buccal Fat Pad in Treatment of Oral Submucous Fibrosis."** submitted by **Dr Shipra Sharma** Post graduate student from the **Department of Oral & Maxillofacial Surgery** as part of MDS Curriculum for the academic year 2018-2021 with the accompanying proforma was reviewed by the Institutional Research Committee present on **26th November 2018** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

Babu Banarasi Das College of Dental Sciences

(A Constituent Institution of Babu Banarasi Das University)

BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Guidelines for Devising a Participant / Legally Acceptable Representative Information Document (PID) in English

1.Study title:

Comparision of the efficacy of Amniotic Membrane v/s Buccal Fat Pad in treatment of Oral Submucous Fibrosis.

2. Invitation Paragraph

You are being invited to take part in a research/trial study. Before you decide, it is important for you to understand why the research/study is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your treating physician/family doctor if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

3. What is the purpose of the study?

The aim of this study is to compare the efficacy of freeze dried irradiated amniotic membrane v/s Buccal Fat Pad as grafting material in surgical management of oral submucous fibrosis.

4. Why have I been chosen?

You are chosen for this study as you are suffering from Oral Submucous Fibrosis with mouth opening < 15mm which is clear indication for surgical interventions and you have not undergone any previous surgery. Total number of 10 patients will be included in this study.

5. Do I have to take part?

It is up to you to decide whether or not to take part in this study. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form and you are still are free to withdraw at any time and without giving any reason.

6. What will happen to me if I take part?

If you take part in this study, preoperative investigations like blood examination, x rays will be taken, and if they are found optimal, you will be included in the

study and also you will be asked to visit us for regular follow through checkup on (2^{nd} day, 1 week, 1 month, 3 month, 6 month) post operatively.

7. What do I have to do?

The ideal protocol will be followed and you would be asked to quit all abusive habits, spicy foods. Do regular physiotherapy for a period of 6 months.

8. What is the procedure that is being tested?

The operative procedure will be carried under general anesthesia. After resection of fibrous bands and bilateral coronoidectomy, the surgical site will be covered by amniotic membrane grafting on one side and BFP on another side and it's effect on the outcome of the surgery will be compared

9. What are the interventions for the study?

It is a surgical intervention to achieve mouth opening.

10. What are the side effects of taking part?

There are no additional side effects.

11. What are the possible disadvantages and risks of taking part?

There are no additional risks associated with this study.

12. What are the possible benefits of taking part?

Improvement of life style and general health as you would gain mouth opening hence proper mastication and good nourishment to the body.

13. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the research being studied. It can be applied in your case if that is beneficial for you as far as treatment outcome is concerned, and that can be done after you have given your consent.

14. What happens when the research study stops?

If the study finishes/stops before the stipulated time, then you would be informed with proper explanation as why the study was stopped.

15. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the complains will be handled by competent person reporting to the institution(s), and IEC. Cost to be bear by personal interest towards treatment in severe adverse event.

16. Will my taking part in this study be kept confidential?

Yes it will be kept confidential.

17. What will happen to the results of the research study?

The result of the study will be published in the indexed journal. Your identity will be kept confidential in case of any report/ publications.

18. Who is organizing the research?

This research study is organized by the candidate and Deaprtment of Oral & Maxillofacial Surgery, BBDCODS.

19. Will the results of the study be made available after study is over?

Yes, only the data obtained will be published.

20. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Department and the IEC of the institution.

21. Contact for further information

Dr. Shipra Sharma
Department of Oral and Maxillofacial Surgery
Shipra4192@gmail.com
BBDCODS, Lucknow.

Dr. Laxmi Bala
Secretary Ethics committee
bbdcods_ice@gmail.com

Name of Principle investigator……

Signature ……..

Date……..

Consent Form (English)

Title of the Study ……..

Study Number……..
Subject's Full Name…….
Date of
Birth/Age
……
Address of the Subject…….
Phone no. and e-mail address……
Qualification ……
Occupation: Student / Self Employed / Service / Housewife/
Other (Please tick as appropriate)
Annual income of the Subject……
Name and of the nominees(s) and his relation to the subject…… (For the purpose of compensation in case of trial related death).

1. I confirm that I have read and understood the Participant Information Document dated ……..for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.
2. I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the project, others working on the Sponsor 's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).
5. I permit the use of stored sample (tooth/tissue/blood) for future research. **Yes [ ] , No [ ] Not Applicable [ ]**

6. I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's Information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative :……..

Signatory 's Name……. Date …….

Signature of the Investigator…… Date……..

Study Investigator 's Name..... Date……..

Signature of the witness…… Date……..

Name of the witness……

Received a signed copy of the PID and duly filled consent form

Signature/thumb impression of the subject or legally Date……..

Acceptable representative

सहमति पत्र

अध्ययन शीर्षक.....
अध्ययन संख्या.....
प्रतिभागी के पूर्ण नाम.....
जन्म तिथि / आयु.....
प्रतिभागी का पता .....
फोन नं. और ई-मेल पता .....
योग्यता .....
व्यवसाय: छात्र / स्व कार्यरत / सेवा / ग्रहिणी .....
अन्य (उचित रुप मे टिक करें).....
प्रतिभागी की वार्षिक आय .....
प्रत्याशीयो के नाम और प्रतिभागी से संबंध...(परीक्षण से संबंधित मौत के मामले मे मुआवजे के प्रयोजन के लिए)

1. मेरी पुष्टि है कि मैने अध्ययन हेतु सुचना पत्र दिनांक .....को पढ व समझ लिया तथा मुझे प्रश्न पुछने या मुझे अध्ययन अन्वेषक ने सभी तथ्यों को समझा दिया है तथा मुझे प्रश्न पुछने के समान अवसर प्रदान किए गये।

2. मैंने यहॉ समझ लिया कि अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है और किसी भी दबाव के बिना स्वतंत्र इच्छा के साथ दिया है किसी भी समय किसी भी कारण के बिना , मेरे इलाज या कानूनी अधिकारो को प्रभावित किए बिना , अध्ययन में भाग न लेने के लिए स्वतंत्र हुॅ ।

3. मैंने यह समझ लिया है कि अध्ययन के प्रायोजक , प्रायोजक की तरफ से काम करने वाले लोग, आचार समिति और नियामक अधिकारियों को मेरे स्वास्थ्य रिकार्ड को वर्तमान अध्ययन या आगे के अध्ययन के सन्दर्भ देखने के लिए मेरी अनुमति की जरूरत नही है, चाहे मैने इस अध्ययन से नाम वापस ले लिया है। हॉलाकि मै यह समझता हुॅ कि मेरी पहचान को किसी भी तीसरे पक्ष या प्रकाशित माध्यम में नही दी जायेगी।

4. मै इससे सहमत हूॅ कि कोई भी डेटा या परिणाम जो इस अध्ययन से प्राप्त होता है उसका वैज्ञानिक उद्देश्य (ओं) के उपयोग के लिए मेरी तरफ से कोई प्रतिबंध नही है।
5. भविष्य के अनुसंधान के लिए भंडारित नमूना (ऊतक/रक्त) पर अध्ययन के लिए अपनी सहमति देता हुॅ।
हॉ [] नही [ ] अनउपयुक्त [ ]

6. मै परीक्षण की अनुमति देता हुं। मुझे इसके द्वारा यदि कोई परेशानी होती है, इसके बारे मे जानकारी दे दी गई है। मैने रोगी जानकारी सूचना पत्र को पढ तथा समझ लिया है।
प्रतिभागी / कानूनी तौर पर स्वीकार्य प्रतिनिधि का हस्ताक्षर (या अंगूठे का निशान.....
.....
हस्ताक्षरकर्ता का नाम..... दिनांक .....
अन्वेषक के हस्ताक्षर ..... दिनांक .....
अध्ययन अन्वेषक का नाम .....
गवाह के हस्ताक्षर ..... दिनांक .....
गवाह के नाम .....
..
मैनें पीआईडी और विधिवत भरे सहमति फार्म का एक हस्ताक्षर की नकल प्राप्त की.

प्रतिभागी कानूनी तौर पर प्रतिनिधि का हस्ताक्षर/ अंगूठे का निशान ..... दिनांक.....
..

CASE HISTORY

Name -	**Age - yrs**	**Sex- M / F**
OPD No. -	**Date -**	
Address -	**Contact No. -**	
Chief complaint -		
History of present illness -		
History of surgery/ Conservative management -		

Medical history	**Dental history**

Extra oral examination – Facial symmetry	TMJ movements	Lymph node

Intra oral examination –

MOUTH OPENING- mm.

TEETH PRESENT-

OCCLUSION -

SOFT TISSUE EXAMINATION-

TONGUE/ LIPS-
HARD AND SOFT PALATE-
BUCCAL MUCOSA-
UVULA-

Investigations –
Radiological-
OPG, PA view of chest

Routine blood investigations –

Hb % -	BT -	CT –			
TLC –	DLC	N-	L-	E-	M-
ESR -	HIV -	HBsAg –			
S.Urea –	S.Creatinine –				
Blood sugar	Fasting -	PP –			

Diagnosis –

Treatment Plan –

Pre-operative Record -

MMO –

RIGHT BUCCAL MUCOSA-

LEFT BUCCAL MUCOSA-

Post-operative Record –			**1st Week**	**1st Month**	**3rd Month**	**6th Month**
	MMO					
	PAIN					
	BURNING SENSATION	**LT SIDE BFP**				
		RT SIDE AM				
	SWELLING	**LT SIDE BFP**				
		RT SIDE AM				
	INFECTION	**LT SIDE BFP**				
		RT SIDE AM				
	SUPPLENESS	**LT SIDE BFP**				
		RT SIDE AM				
	HEALING	**LT SIDE BFP**				
		RT SIDE AM				
	PIGMETATION	**LT SIDE BFP**				
		RT SIDE AM				

Complication if any:-

Urkund Analysis Result

Analysed Document: shipra merged.pdf (D110196080)
Submitted: 7/6/2021 11:25:00 AM
Submitted By: hemantmehra121@bbdu.ac.in
Significance: 8 %

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