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EVALUATION OF COLLAGEN IN HEALING OF MUCOSAL DEFECTS AFTER VESTIBULOPLASTY: A CLINICAL STUDY

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ABSTRACT

Background and objectives: Grafts are used in vestibuloplasty to avoid relapse due to secondary wound contracture as seen with secondary epithelization technique. Conventional methods like free mucosal grafts and split thickness grafts require the creation of second surgical wounds for use as donor site. Collagen is a biological skin substitute, i.e. natural, easily available, ready to use, has excellent tissue compatibility, non-immunogenic, and non-pyrogenic. Commercially available collagen sheets are used in the treatment of burns, ulcers, diabetic ulcers, bed sores etc as a biological dressing material. The main aim of the study was to evaluate clinically the efficacy of collagen sheet as replacement in the vestibular extension instead of mucosal or skin grafts. **Methodology**: 15 edentulous patients requiring vestibular extension procedure were included in this study. Clark's secondary epithelization vestibuloplasty technique was followed in our study and the exposed periosteum was covered by collagen sheet and stabilized by surgical splint. Postoperative evaluation (after 24, 48 and 72 hours followed by 1st, 2nd and 3rd week) included efficacy of collagen as a temporary biological dressing material, promotion of wound re-epithelization, prevention of wound contracture and reduction of pain and swelling. **Results:** The post-operative course of all 15 patients was uneventfull. By the end of first week post-operatively, lysis of collagen sheet was evident with underlying hyperemic tissue. Healthy granulation tissue covered the entire surgical wound by the end of 2nd week and epithelization was complete by 3rd week. All patients had good hemostasis and marginal wound contraction. Most of the patients complained of only mild pain. The post-operative swelling was significant in all patients. **Conclusion**: Collagen sheet might be used as a potential graft material for vestibuloplasty.

KEYWORDS: Collagen sheet; collagen membrane; graft; artificial dermis; vestibuloplasty.

INTRODUCTION

The preprosthetic manipulation of oral soft and hard tissues is an accepted modality of treatment in bringing about oral rehabilitation of aesthetics and function in the edentulous patients. Vestibuloplasty has become the most popular surgical method for improving the denture retentive and stabilizing capabilities of the alveolar ridge. The technique is simple and makes no attempt to cure the alveolar atrophy; rather it attempts to expose and make available for denture construction that bone which is still present, to provide a base of healthy, firmly attached alveolar mucosa.[1]

Various vestibular extension techniques for treating the edentulous patient have been described since 1935.[2] The three basic techniques for vestibular extension are submucosal vestibuloplasty, secondary epithelization vestibuloplasty, and soft tissue grafting vestibuloplasty.[2-3] Secondary epithelization was found to be ineffective because the postoperative vestibular depth often decreased over the time as a result of wound contraction.[4] Propper was the first to report that contracture of the extended area had been prevented by addition of tissue rather than mobilization of adjacent tissues.[5]

The employment of skin grafts in oral vestibular sulcus extension surgery has a long evolutionary history of success.[6] Skin grafts have disadvantages, such as lack of denture adhesion, the presence of hair, contraction of the graft, and an extensive wound in skin.[7] Palatal mucosal grafts are the ideal grafts for the oral cavity. The major disadvantages of palatal mucosal grafts are donor area morbidity and limited size.[4]

To compensate for these disadvantages of skin and mucosal grafts, various graft materials like amnion[7], lyophilized dura[8], collagen, porcine skin and synthetic plastics have been tried in vestibular extension procedures.[9]

A biological covering is advantageous in areas denuded of an epithelial lining to protect the area while epithelial regeneration occurs, to resist bacterial growth on the surface of tissue and to minimize wound contracture.[10]

A resorbable, naturally occurring substance, collagen has been incorporated into a variety of medical devices and has been used for multiple purposes. Collagens have been used as a hemostatic agent and biological dressing, as well as in the management of burn wounds, in conjunction with ophthalmologic and orthopedic procedures, and for oral, dental, and plastic surgeries. Dermatologic and cosmetic surgeons have used collagen for soft-tissue augmentation. In addition to their usefulness for skin augmentation and as a dressing, several types of vascular prostheses have been derived from collagen.

For oral applications, homogenized reconstituted collagen mixed with cell culture media has been used for burn treatment and for endodontic repair. Resorbable collagen wound dressings have been used in oral wounds and closure of grafted areas or extraction sites because, they stabilize blood clots, protect surgical sites, and accelerate the healing process. Perhaps more importantly, collagen-based membranes have been widely used in periodontal and implant therapy as barriers, that prevents the migration of epithelial cells and encourage wound repopulation by cells with regenerative potential.[11]

The objective of present study was clinical evaluation of the role of collagen in healing of mucosal defects after vestibuloplasty done under general anesthesia; for patients who have moderate to severe alveolar ridge atropy leading to a functional oral handicap and improper prosthetic rehabilitation of oral function.

MATERIAL AND METHODS

A study was conducted on 15 edentulous patients, required vestibular extension procedures in the mandibular arch. Out of 15 patients 5 were females and 10 were males. The age of the patients ranged from 22 years to 70 years. A standard proforma was used to select and evaluate the cases.

INCLUSION CRITERIA

1. Patients with a minimal residual bone height of 10mm.
2. High soft tissue attachments that prevent adequate flange extension for denture stabilization, which includes high mucosal, frenal and muscle attachments.
3. Redundant soft tissue on ridge and vestibulum.

Exclusion criteria

1. Severe resorption of basal bone, with residual bone height less than 10mm.
2. Jaws with underlying disease.

Out of 15 patients, only 2 patients were denture wearers and remaining 13 patients never had dentures before.

Pre operative assessment

General and systemic conditions were evaluated to know their fitness to undergo the surgery. Routine hematological investigations were done for every patient and Orthopantomograms were taken to determine the size and shape of alveolar base and location of mental foramen.

Pre-operatively, a transparent acrylic plate with overextended flange borders was fabricated on a stone cast of a mandibular ridge alginate impression. The pre-operative sulcus depth was measured by sectioning the cast and measuring with a caliper for statistical records.

Among the 15 patients, 3 patients required vestibuloplasty from canine to canine region, 7 patients from premolar to premolar region and 5 patients from molar to molar region in the mandible. Possible vestibular extension was determined by bimanual palpation and model studies.

Collagen sheet

In all patients, in this study, the exposed periosteum was covered with collagen in the form of sheets, which was enzymatically prepared from cattle serosa. The collagen sheet used was a branded material Kollagen, manufactured and marketed by Eucare pharmaceuticals (P) Ltd, Chennai. The manufacture supplied sterile collagen sheet in preserving medium containing isopropyl alcohol and water and sterilized by gamma irradiation. Collagen sheet of dimensions of either 5x5 cm or 10x10 cm was used depending upon the requirement.

Anaesthesia

All patients underwent the surgery under general anaesthesia. After securing general anaesthesia, the lower lip was hyperextended and local anaesthetic solution containing 2% lignocaine hydrochloride with 1: 200000 adrenaline was infiltered in vestibule for proper submucosal dissection and good hemostasis.

Surgical procedure

All surgeries were carried out under strict aseptic precautions. Injection Amoxicillin-1g and injection Dexona 8mg was administered intravenously as prophylactic drugs for all patients. Clark's technique of vestibuloplasty was used in this study. An incision was made at the junction of free mucosa and attached gingiva using no. 15 blade extending from the region of canine to canine or premolar to premolar or molar to molar depending on areas indicated. A supraperiosteal flap was dissected with sharp scissors. All muscle and loose

connective tissue were removed from the periosteal surface. As some regression was expected maximal exposure of alveolar bone was achieved. When the ridge was adequately exposed, collagen sheet after thoroughly rinsing with normal saline was spread over the exposed periosteum and was cut to the required size. The collagen membrane was sutured to the labial flap and the periosteum using 4-0 vicryl at the newly created vestibular depth by placing simple interrupted sutures. Superiorly the collagen membrane was sutured to the attached gingiva using 4-0 vicryl and placing simple interrupted sutures.

Splint stabilization

After placement of sutures, the area was covered with the transparent acrylic plate. The transparent acrylic plate was lined by either tissue conditioner or soft liner in order to avoid irritation to collagen sheet and eliminate the dead space. The acrylic splint was immobilized over the collagen with circummandibular wiring. For circummandibular wiring, a bone awl was passed through the skin beneath the chin and up into the mouth on the lingual side of mandible. A length of 26 G stainless steel wire was threaded to its tip and the awl was withdrawn to the lower border of mandible. Keeping the awl close to the bone it was passed around the lower border and then pushed into the buccal sulcus. The wire was detached from the tip of the awl and the instrument was then withdrawn from the tissues through the external wound. The two free ends on each side were twisted together over the splint, cut short and bent to form cleats.

Post operative care

All patients were admitted for 5 days postoperatively and the following drugs were administered

1. Inj. Amoxicillin 500mg, 1 tid x 5 days.
2. Inj. Metrogyl 100ml, 1 tid x 5 days
3. Inj. Voveran, 1 bid x 3 days.

Patients were advised to have liquid or semisolid diet and to rinse their mouth using chlorhexidine mouthwash. The acrylic stent was removed 1 week after surgery and suture removal done after 2 weeks.

Post operative assessment

All patients were evaluated after 24 hours, 48 hours and 72 hours followed by the end of 1st week, 2nd week, 3rd week, 1 month, 2 months and 3 months.

Hemostatic effect was assessed on the 1st post operative day as good (no bleeding), fair (slight bleeding, no haemostasis required) and poor (bleeding that required hemostasis).

Pain and swelling were assessed postoperatively after 24 hours, 48 hours and 72 hours.

Pain

Pain being a subjective symptom was assessed using verbal analogue scale, ranging from 0 (none) to 10 (agonizing) as No pain (0), mild (1-3), moderate (4-6) and severe (7-10).

Swelling

Both preoperative and postoperative measurements recorded from right angle of the mandible to the left angle of the mandible passing on a fixed point on the symphysis and from right angle of the mouth to a point on the symphysis and to the left angle of the mouth were compared.

Allergic manifestations

Allergic manifestations to collagen were evaluated by inspecting intra orally for local allergic reactions such as erythema, wheal etc and general examination for rashes, itching, pruritis, urticaria etc.

Condition of collagen

Was assessed as healthy collagen (1) and sloughing of collagen (2), after the removal of the acrylic splint at the end of 1st week. The degree of granulation tissue formation and re-epithelization was evaluated upto 1 month postoperatively. Granulation tissue formation was assessed as 0 (no granulation tissue), + (wound margins covered with granulation tissue), ++ (complete wound covered with granulation tissue), +++ (granulation tissue above wound bed) and ++++ (wound and margins covered with granulation tissue). Re-epithelization was assessed as 0 (epithelization not evident), + (nearly the entire wound) and ++ (entire wound) By the end of 4th week postoperatively, all the patients were referred to Department of Prosthodontics for fabrication of complete dentures. At the end of 1 month, 2 months and 3 months, impressions were made and cast prepared as done preoperatively and the sulcus depth measured to assess the net gain in sulcus depth and wound contracture.

RESULTS

In this study, a total of 15 patients with insufficient vestibular depth in the mandibular labial sulcus were treated by Clark's technique of vestibuloplasty and the raw periosteum was covered with collagen sheet and acrylic stent stabilized with circummandibular wiring under general anaesthesia. Among the 15 patients, 10 patients were men and 5 women, ranging from age 22 to 70 years with mean age of 54.7 years {T-1&2\ G-1&2}. On classifying the alveolar ridges and stage of edentulous bone loss of the subjects, 2 patients belonged to Class I stage I a, 6 patients to class I stage II a, 2 patients to Class II stage I a, 2 patients to Class II stage II a and 3 patients to Class III stage III a { T-3\ G-3}. The acrylic stent was removed after 1 week in all patients.

All patients were evaluated for efficacy of collagen sheet as a temporary biologic dressing material, wound re-epithelization, wound contraction, pain and swelling. During the statistical analysis, p values of <0.05 or <0.01 were considered as significant; while a p value of <0.001 suggested highly significant value and p >0.05 was considered statistically insignificant.

Haemostatic effect was assessed on the 1st post operative day as good, fair and poor. In all patients in this study,

hemostatic effect was found to be good, suggesting no post operative bleeding.

Pain being a subjective symptom was assessed using verbal rating scale and rated as mild, moderate and severe as described by the patients. In this study 12 patients complained of mild pain, 2 patients had moderate pain and only 1 patient complained of severe pain { T-4\ G-4}.

Statistically significant post-operative swelling was evident on day 1, 2 and 3 (ANOVA test). Statistical analyses of pre-operative measurements with post operative measurements were highly significant between the pairs pre-operative and day1, pre-operative and day 2 and pre-operative and day 3 (Student Newman Keul's test) {T-5\ G-5}.

Allergic manifestations to collagen were evaluated by inspecting intra orally for local allergic reactions such as erythema, wheal etc and general manifestations like rashes, itching, pruritus, urticaria etc and no allergic response was evident in all the 15 subjects.

Following the removal of the splint a week after the surgery, sloughed and disintegrating collagen with underlying hyperemic tissue was evident. By the end of second week there was no evidence of collagen membrane and the surgical wound was covered by granulation tissue. The condition of the collagen sheet at the end of 1st week and 2nd week on statistical analysis was found to be highly significant on all the patients [p <0.001] (Chi square test) {T-6\ G-6}.

The wound margins were covered with granulation tissue in all the patients by the end of 1st week and complete wound was covered with healthy granulation tissue by the end of 2nd week in 13 patients, and healthy granulation tissue growing above the wound bed was evident in 2 patients, which are statistically found to be highly significant [p <0.001] (Chi square test) {T-7\ G-7}.

At two weeks post-operatively, the area of operation was seen to be covered by a thin epithelial layer and the normal appearance of the area of operation was restored by the end of 3rd week post-operatively with smooth, newly synthesized oral mucosa in all the patients, which was statistically highly significant [p <0.001] (Chi square test) {T-8\ G-8}.

The vestibular depth was measured pre operatively, 1 month and at of 3 months post-operatively. The mean post operative depth was 4.53 mm with a S.D of +\-1.13. The mean 1 month post-operative vestibular depth was 11.4 mm+\-1.88 (S.D) and the same at the end of 3 months was 11.33mm+\-1.99 (S.D) and statistically significant (ANOVA test). The statistical analysis was highly significant between the pairs pre operative and 1 month post-operative and between pre-operative and 3 months post-operatively in all the patients (Student Newman Keul's test) {T-9\ G-9}.

The net gain in sulcus depth achieved at the end of 1 month was 6.87mm and at the end of 3 months was 6.8mm. the net gain in sulcus depth at the end of three months was 6.8+\- 1.61.the wound contracture at the end of 3 months was 0.07mm.

Post-operative wound contraction was found to be minimal at the end of 3 months. No complications such as immunologic rejection and infection occurred in any of the patients. Only one patient reported transient parasthesia on the left side of the lower lip.

Table 1: Demographic variables.

Age (Years)	No of Cases (%)
21-30	1 (7)
31-40	0
41-50	3 (20)
51-60	6 (40)
61-70	5 (33)
Total	15 (100)
Mean +/-SD	54.7+/-12

Table 2: Sex Distribution.

Gender	No of Cases (%)
Male	10 (67)
Female	5 (33)

Table 3: Diagnosis.

Diagnosis	Stage Ia	Stage IIa	Stage IIIa
Class I	2 (13)	6 (40)	0
Class II	2 (13)	2 (13)	0
Class III	0	0	3 (20)

Table 4: Pain intensity score.

Pain	Mild	Moderate	Severe
Day 1	12 (80)	2 (13)	1 (7)
Day 2	12 (80)	2 (13)	1 (7)
Day 3	12 (80)	2 (13)	1 (7)

Table 5: Swelling.

Swelling	Pre op (Mean ± SD)	Day 1 (Mean ± SD)	Day 2 (Mean ±SD)	Day 3 (Mean ± SD)	P* Value, Sig	Significant pairs**
a	14.16±1.59	15.63 ± 1.5	15.63 ±1.53	15.63 ±1.5	P<0.001 HS	I&II, I&III,I&IV
b	22 ± 2.1	24 ± 2.1	24 ± 2.1	24 ± 2.1	P<0.001 HS	I&II, I&III,I&IV

* Repeated measures ANOVA test

** Student Newman Keul's test

Table 6: Condition of the graft.

Condition	Not evident	2
1st week	0	15 (100)
2nd week	15 (100)	0

$X^2 = 26.3$ P<0.001 HS

Table 7: Granulation Tissue.

Granulation Tissue	Week 1	Week 2
+	15 (100)	0
+ +	0	13 (87)
3 +	0	2 (13)

$X^2 = 30$ P<0.001 HS
$X^2 = 120$ P<0.001 HS

Table 8: Re-Epithelization.

Re-Epithelization	0	+	+ +
1st week	15 (100)	0	0
2nd week	0	15 (100)	0
3rd week	0	0	15 (100)
4th week	0	0	15 (100)

Table 9: Sulcus depth.

Sulcus depth	Mean+/-SD	P* Value, Sig	Significant pairs**
Pre-Operative	4.53+/-1.13	P<0.001 HS	I & II, I & III
1 Month Post-operative	11.4+/-1.88		
3 Months Post-Operative	11.33+/-1.99		

* Repeated measures ANOVA test
** Student Newman Keul's test

DISCUSSION

Collagen materials have been utilized in medicine and dentistry because of their proven biocompatibility and capability of promoting wound healing.[30,31] Collagen products have been utilized as major components of artificial tissue substitutes, which have to be biocompatible and biodegradable, after the tissue damage has regenerated and healed. Recently, artificial collagen grafts have been used in the repair of soft tissue defects or burn wounds, as temporary skin substitutes.[12]

In this study, collagen was used in the form of sheets, manufactured and marketed by Eucare pharmaceuticals (P) Ltd, Chennai as Kollagen. Collagen sheets were produced from cattle serosa comprising mostly of type I and III collagen in a preserving medium containing isopropyl alcohol and water and sterilized by gamma irradiation. Irradiation has 2 main effects on collagen: initiating random crosslinks and breaking the tropocollagen molecule.[11]

The present study was designed to evaluate the clinical efficacy of collagen sheet as a biologic dressing material, in wound reepithelization and preventing wound contracture when used as a mucosal substitute after vestibuloplasty.

Preprosthetic oral surgery emerged from a ridge trimming service to a truly reconstructive service when Kazanjian (1924, 1935) reported on the prototype of labiobuccal vestibuloplasty procedures. His technique was modified by Godwin (1947), Clark (1953) and others.[3] In both Kazanjian and Clark's techniques of vestibuloplasty, there was obliteration of the artificially created new sulcus by contracture[13], resulting in considerable regression in vestibular depth and no significant difference between the degrees of loss in the two procedures was observed.[14]

In this study we followed Clark's technique of secondary epithelization vestibuloplasty. It is widely accepted that skin grafts have an excellent capacity for maintaining a surgically created vestibular extension. Healthy skin grafts largely retain their pre-grafting clinical and histological characteristics. The disadvantages of denture bearing skin grafts are lower response threshold to denture trauma, higher susceptibility to Candida infection, contraction of graft, lack of denture adhesion, presence of extensive graft site wound and hair growth.[6,8]

Collagen sheet has been found to be well tolerated in clinical trials. There have been no reports of clinically significant immunological or histological responses to the implementation of collagen sheet, and no reports of rejection of collagen sheet.[15]

Before healing can begin, hemostasis must occur. In this study; haemostatic effects of collagen were evaluated as good, fair and poor on the 1st post operative day. Haemostsis was good in all patients, suggesting no bleeding. The collagen sheet absorbed blood and exudates immediately after attachment. The resulting platelet agglutination and blood clot promoting effects may have been effective.[16] Collagen applied at the time of haemostasis acts as an auxiliary mechanism to augment clotting. Collagen actually increases platelet adherence to the endothelial vessel wall, thus sealing it off.[15]

The degree of pain was assessed post-operatively for 24 hours, 48 hours and 72 hours as mild, moderate and severe. In this study 12 patients complained of mild pain, 2 patients had moderate pain and only 1 patient complained of severe pain. Collagen when used to cover raw exposed wounds provides protection from the stimuli by covering the sensitive nerve endings, there by

diminishing the degree of pain.[9,16,17,18] Those patients with severe pain can be attributed to the post surgical traumatic inflammation.

Observations were made for three days post-operatively and measurements were recorded for 24, 48 and 72 hours. When these values were statistically analysed with pre-operative measurements, the post-operative swelling was statistically significant. In a similar study, the post-operative swelling was not found to be significant[9].

In this study, no local or general allergic reactions were evident in all the 15 patients. Hence the antigenicity of the collagen sheet was regarded as not significant.

Collagen has been demonstrated to be a weak immunogen and is therefore well tolerated by patients. A study by Johns et al demonstrated that membranes made of bovine collagen do not elicit an antibody response when used in GTR. This is not a surprising finding given the extensive distribution of collagen throughout the body.[18]

Because bovine collagen is the main constituent in this sheet, antigenicity and the possibility of hypersensitivity to this sheet must be considered. Its telopeptide is the main expression site of collagen antigenicity.[16] Studies have shown that, when the collagen is treated by UV or gamma radiation, immunologic reactivity is lost.[11-12] Collagen sheet used in our study was sterilized by gamma radiation. Thus antigenicity may not be a problem and was not detected in this study.

Few investigators have shown that mechanical stents can effectively limit wound contraction.[19] In this study, we always used a resin plate to splint the dressing template as well as tissue conditioner or soft liner for appropriate even compression and adhesion, as suggested by other authors.[16]

The wound margins were covered with granulation tissue in all the patients by the end of 1st week and complete wound was covered with healthy granulation tissue by the end of 2nd week in 13 patients and healthy granulation tissue growing above the wound bed was evident in 2 patients. At two weeks post-operatively, the area of operation was seen to be covered by a thin epithelial layer and the normal appearance of the area of operation was restored by the end of 3rd week post-operatively with smooth, newly synthesized oral mucosa in all the patients. These findings were statistically significant and correlated with the findings of other authors.[9-10]

Collagen serves as a template for the infiltration of fibroblasts, macrophages, and lymphocytes and attracts additional monocytes to the wound, thus increasing the amount of debris removed and capillaries forming the neovascular network. As healing progresses, collagen is deposited by the fibroblasts, replacing the collagen portion of the collagen sheet.[20]

The net gain in sulcus depth achieved at the end of 3 the months was 6.8+\-1.61mm and the wound contracture at the same time was found to be marginal. In order to prevent relapse all patients were referred to Department of Prosthodontics for early fabrication of complete denture with extended labiobuccal flange.

Studies done by Starshak and Sanders resulted in 50 % relapse only after one year, but Laskin et al noted only 19 % relapse in mandibular vestibuloplasty.[3] Kasper and Laskin have demonstrated that collagen fibers in wound contraction were oriented in an anteroposterior, or horizontal direction and major forces acting on the wound margins were in this direction, resulting in relapse.[19]

Out of the 15 patients in our study only one patient had transient paresthesia of the left lower half of the lip and one patient developed subcutaneous abscess in the submental region where the bone awl was passed for circummandibular wiring. Both these complications can be attributed to surgical procedure and not due to the collagen sheet. There was no immunogenic rejection or infection to the collagen sheet in our study. None of the patient complained of altered taste, smell with regards to the presence of graft.

In this study, there is no means of determining if the collagen sheet actually entered into the healing process, contributing its triple helix structure for regenerating tissue or does it function strictly as a biologic dressing? In either case the collagen sheet certainly did not appear to delay healing. Further research needs to be carried out, which would throw light on the role of collagen, and its outcome at the histological level when used clinically.

In these cases we were able to demonstrate that the use of collagen sheet we could achieve tissue regeneration instead of using tissue transplantation. This therapeutic concept could lead in selected patients in the future to better cosmetic results, shorter treatment time and less donor site morbidity.

CONCLUSION

In this study, we evaluated the collagen sheet originally developed for covering dermal defects in patients undergoing plastic surgery for its application in oral surgery. We evaluated the effectiveness of collagen sheet for vestibular extension by assessing haemostatic effects, pain relief, granulation, reepithelization and contracture on 15 patients, out of which 10 patients were men and 5 women, ranging between 22 to 70 years.

Within the limits described in this study, the following conclusions can be statedCollagen sheet provides an excellent alternative to Autogenous soft tissue grafting, esp. in cases similar to this study, in which large areas of

keratinized tissues are needed. Approach is an effective alternative in patient requiring vestibuloplasty before fabrication of dentures.

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