Trends in Biomaterials and Artificial Organs
sbaoi
Original Article
www.sbaoi.org/tibao

Effectiveness of Chitosan versus Collagen Membrane for Wound Healing in Maxillofacial Soft Tissue Defects: A Comparative Clinical Study

Ahmed Elham Haque*, K. Ranganath, Kavitha Prasad, Sejal Kumarpal Munoyath, R.M. Lalitha

Department of Oral and Maxillofacial Surgery, Ramaiah University of Applied Sciences, Gnanagangothri Campus, Bangalore 560054, India

Received 6 August 2019
Accepted 30 September 2019
Published online 31 March 2020

Chitosan has been shown to act as an effective hemostat and antimicrobial agent with additional aid in wound healing. The purpose of this study was to compare the wound healing properties of Chitosan versus a more established material like Collagen in a clinical controlled trial. A study conducted at our institution, encompassed all the patients with facial soft tissue abrasions, from July 2016 to July 2017. They were randomly divided into two groups, half treated with Chitosan membrane and the other half treated with Collagen. Comparisons were made according to the pain, time taken for granulation tissue formation and scarring. Sixty patients were included in the study. It was observed that pain reduction over a week was significantly higher in the Collagen group than in the Chitosan group. Significant results in favor of Chitosan were found with regard to average time taken for granulation tissue formation and pigmentation of the resultant scars. The results indicate that Chitosan enhances soft tissue healing, improves color matching and minimizes scarring, as compared to Collagen. Chitosan in its non-resorbable form, however required multiple dressing changes. Chitosan membrane can be an alternative to Collagen membrane as a facial wound dressing material.

Introduction

Wound healing is a specific biological process related to the general phenomenon of growth and tissue regeneration [1]. The variety of wound types has resulted in a wide range of wound dressing materials with new products frequently introduced to target different aspects of the wound healing process. Wound dressings and devices form an important segment of the medical and pharmaceutical market. The ideal dressing should achieve rapid healing at reasonable cost with minimal inconvenience to the patient. In the past, traditional dressings such as natural or synthetic bandages, cotton wool, lint and gauzes all with varying degrees of absorbency were used for the management of wounds. Modern dressings are based on the concept of creating an optimum environment to allow epithelial cells to move unimpeded, for the treatment of wounds. Such optimum conditions include a moist environment around the wound, effective oxygen circulation to aid regenerating cells and tissues and a low bacterial load [2]. It is important therefore, that different dressings be evaluated and tested in terms of their physical properties and clinical performance for a given type of wound and the stage of wound healing, before being considered for routine use.

While a few materials (e.g.: collagen, PRFM, amniotic membrane) have gained popularity in recent times and are being used on a regular basis in management of facial wounds, other materials (eg: chitosan) are being introduced into the market and require further studies on humans to justify their use. Chitosan, has in recent times shown to act as an effective hemostat and antimicrobial agent with additional aid in wound healing. Chitosan is a β-1,4-linked polymer of glucosamine (2-amino-2-deoxy-β-D-glucose) and lesser amounts of N-acetylglucosamine. It is a derivative of chitin (poly-Nacetylglucosamine), found in mushrooms, crustaceans (such as crabs, lobsters, shellfish and shrimp), in the radulas of mollusks, and the beaks of cephalopods (including squid and octopuses) [3]. It is also commercially readily available and cost-effective. Literature search provides very few articles evaluating and comparing the use of chitosan dressings with other materials in the head and neck region. The purpose of this study was to compare the wound healing properties of chitosan versus a more established material like collagen in a clinical controlled trial.

* *Coresponding author*
E-mail address: elhamhaque@gmail.com (Department of Oral and Maxillofacial Surgery, Ramaiah University of Applied Sciences, Gnanagangothri Campus, New BEL Road, MSR Nagar, Bangalore 560054, India)

Materials and Methods

A controlled clinical trial was performed on all patients with facial soft tissue injuries (abrasions), reporting to the Accident & Emergency Department of Ramaiah Memorial Hospital, Ramaiah Medical Teaching Hospital and Department of Oral and Maxillofacial Surgery Faculty of Dental Sciences, Ramaiah University of Applied Sciences between July 2016 and July 2017. They were randomly divided into two groups, with half the patients treated with Chitosan Membrane and the other half patients treated with Collagen Membrane. Patients were selected prospectively and Randomization of the study was done using allocation concealment while determining the choice of dressing material. Sequentially numbered, opaque, sealed envelopes were used as the allocation concealment scheme. Each envelope contained the names of either one of the materials (chitosan/collagen) and was handed to the operator by a blinded investigator. The operator applied the dressing material given to him as per the manufacturer's instructions. Patients in both groups were prescribed oral NSAIDS (in the form of Aceclofenac+Paracetamol), for 3 days after the initial application of the dressing, to alleviate pain. Any rescue medications taken by the patient after 3 days were also noted. Each patient was handed a proforma portraying the Visual Analog Scale. This was handed to the investigator in proceeding follow ups. Blinding, at this stage, was not possible on account of the difference in the frequency of dressing changes between the two materials. Other parameters, such as wound healing and scar judgement, were assessed based on the mean observations made by the two investigators. Wounds with significant amount of tissue loss (>2mm depth) were given a supplemental dressing in the form of L - Platelet Rich Fibrin (L-PRF) membrane prior to the dressing in both groups. The wounds were classified as 'Superficial' and 'Deep' based on the amount of tissue loss (even on the same patient).

Inclusion Criteria

Patients with facial soft tissue injuries/abrasions

Exclusion Criteria

Medically Compromised Patients, i.e., patients with bleeding disorders, immunocompromised patients (diabetes mellitus, patients on corticosteroid therapy).

Physically or intellectually impaired patients and patients unable to respond to questions

Patients with Extensively Deep wounds / Full-Thickness Wounds/ Infected Wounds / Wounds with tissue loss.

Patients with superficial soft tissue loss in the facial region and who require dressing material for wound coverage were included in the study. Patients were randomly allocated to either group to avoid bias.

Figure 1: (a) Chitosan (Axiostat); (b) Collagen (Kollagen)

Chitosan membrane is commercially available as Axiostat, which is 100% chitosan derived from shell of crustaceans. Axiostat used in this study was of 2 sizes, 5 x 5 cm and 8 x 8 cm. The commercially available collagen membrane (Kollagen) which is a bovine derived collagen, was used in this study. Kollagen used in this study was of 2 sizes, 5 x 5 cm and 8 x 8cm. Both materials were supplied in sterile preserving medium, Gamma sterilized and available in different dimensions.

Allocation was done to either Group A (Chitosan Group) or Group B (Collagen group)

Group A (Chitosan Group): The wound was washed with normal saline and betadine solution. Under strict aseptic precautions, the wound was dried, the dressing material cut into desirable size and applied over the wound. The membrane adhered to the wound and a gauze dressing placed over the membrane to hold it in place. The dressing was assessed and changed every 2 days as advised by the manufacturer

Group B (Collagen Group): The wound was washed with normal saline and betadine solution. Under strict aseptic precautions, the collagen membrane was soaked in normal saline to wash off the preservatives, cut into desirable size and applied to the wound.

Both groups of patients were observed twice in the first week, with subsequent follow ups on the 2nd week, 3rd week, 1 month and 2 months.

Scoring and data analysis

Pain: Relative Pain Scores were assessed based on the patient's own

Table 1: Vancouver Scar Scale

	Feature	Score
Vascularity	Normal	0
	Pink	1
	Red	2
	Purple	3
Pigmentation	Normal	0
	Hypo-pigmentation	1
	Hyper-pigmentation	2
Pliability (Elasticity)	Normal	0
	Supple (flexible with minimal resistance)	1
	Yielding (giving way to pressure)	2
	Firm (inflexible, not easily moved, resistant to manual pressure)	3
	Banding (rope-like tissue that blanches with extension of scar)	4
	Contracture (permanent shortening of scar, producing deformity or distortion)	5
Height	Flat	0
	<2mm	1
	2-5 mm	2
	>5 mm	3

Table 2: Comparison of mean VAS scores for pain between 24 hours & 1 week in Chitosan & Collagen group using Wilcoxon Signed Rank test

Group	Time	N	Mean	SD	Mean Diff	Z	P-Value
Chitosan	24 hours	30	1.40	1.30	0.50	-1.688	0.09
	1 week	30	0.90	1.35			
Collagen	24 hours	30	2.03	1.73	1.30	-3.469	0.001*
	1 week	30	0.73	1.26			

assessment and visual analogue scale from 0 (no pain) to 10 (maximum pain) after 24 hours of applying the dressings. An assessment on day 7 was done when the patient was off medications.

Rate of wound healing was measured by: The number of days required for completion of granulation tissue formation in both groups. This was assessed when the operator deemed the application of further Chitosan dressing as unnecessary in case of Group A or when the Collagen dressing peeled off completely in Group B.

Judgement for scar was done 2 months post-operatively by the blinded investigators based on the score pattern in Vancouver Scar Scale given by Draaijers et al (table 1). The Vancouver scale given by Draaijers et al assesses the scar in 4 domains: vascularity, pigmentation, pliability and height. The scores range from 0 to 13, the maximum score indicating the worst result and 0 score indicating normal skin. Judgement for scar was done 2 months post-operatively by the blinded investigators based on the score pattern in Vancouver Scar Scale.

The Facial region was divided into the following parts so as to assess the most commonly affected areas with soft tissue injuries following trauma and the distribution in the two groups. The regions were divided as: Frontal, Temporal (above the zygomatic arch), Malar (below the zygomatic arch), Nasal, Lips (Upper and Lower), Chin and Auricular. The Ethical Consent for the study was obtained from the Institutional Review Board and Ethics Committee.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Chi Square test was used to compare the areas involved, Vancouver Scar scale responses between Chitosan & Collagen groups. Independent Student t test was used to compare the mean rate of wound healing between Chitosan & Collagen groups. Mann Whitney U test was used to compare the mean VAS scores for pain

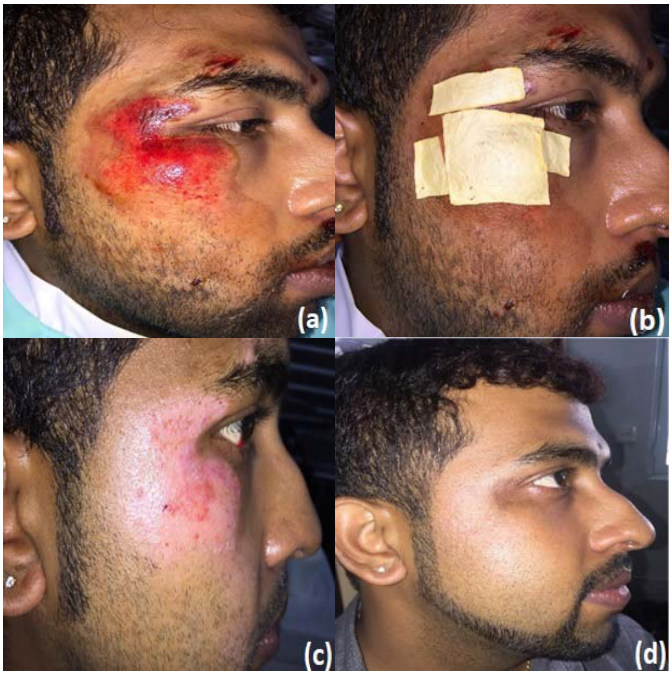

Figure 2: Application of Chitosan (Axiostat) over Superficial Abrasion in Temporal Region; (a) Pre-Application; (b) Post-Application; (c) 8 days post-application; (d) 2 months post-application

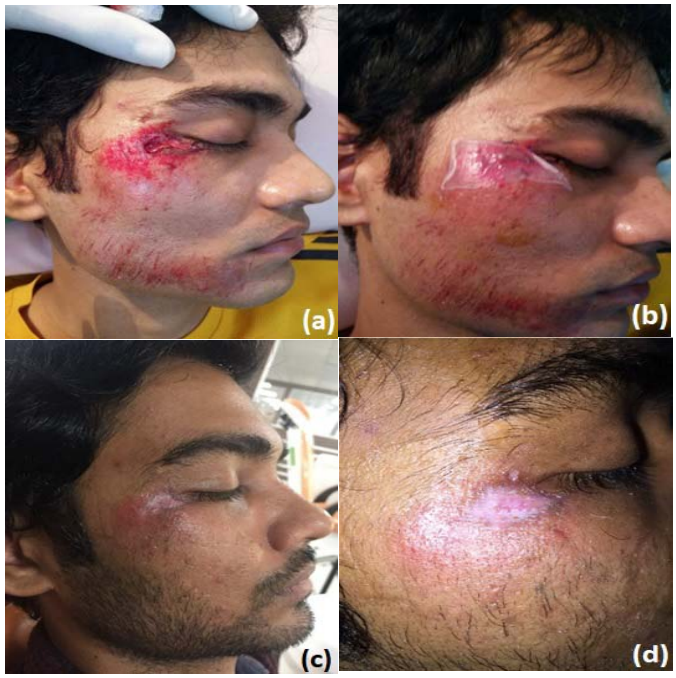

Figure 3: Application of Collagen (Kollagen) over Superficial Abrasion in Temporal Region; (a) Pre-Application; (b) Post-Application; (c) 11 days post-application; (d) 2 months post-application

Table 3: Comparison of Vancouver Scar scale responses between Chitosan & Collagen group using Chi Square test

Scar Scales	Categories	Chitosan		Collagen		c^2 Value	P-Value
		n	%	n	%		
Pigmentation	Normal	17	56.7%	10	33.3%	6.364	0.04*
	Hypopigmented	5	16.7%	14	46.7%		
	Hyperpigmented	8	26.7%	6	20.0%		
Vascularity	Normal	21	70.0%	19	63.3%	0.350	0.84
	Pink	7	23.3%	9	30.0%		
	Red	2	6.7%	2	6.7%		
	Purple	0	0.0%	0	0.0%		
Pliability	Normal	19	63.3%	17	56.7%	3.063	0.55
	Supple	8	26.7%	6	20.0%		
	Yielding	2	6.7%	4	13.3%		
	Firm	0	0.0%	2	6.7%		
	Ropes	1	3.3%	1	3.3%		
	Contracture	0	0.0%	0	0.0%		
Height	Normal	23	76.7%	24	80.0%	4.621	0.10
	1-2 mm	7	23.3%	3	10.0%		
	2-5 mm	0	0.0%	3	10.0%		
	>5 mm	0	0.0%	0	0.0%		

between Chitosan & Collagen groups. Wilcoxon Signed Rank test was used to compare the mean VAS scores for pain between 24 hours & 1 week in Chitosan & Collagen groups. The level of significance [P-Value] was set at $P<0.05$.

Results

Age and Gender Distribution

A total of 60 patients were selected, 30 in each group. The age and gender distribution in both groups was statistically insignificant. Males accounted for nearly two-third the sample size in each group (20 males and 10 females in Group A, 19 males and 11 females in Group B). The age ranged from 17 years to a maximum of 55 years in both groups.

Type of Wounds

The wounds were classified as superficial and deep wounds. Superficial wounds were treated with the prescribed dressings alone whereas the deep wounds were treated for depth with adjuncts such as PRF prior to application of dressing as a barrier or a scaffold. In this study, out of the 60 patients, 7 patients in both groups presented with deep wounds.

Areas Involved

The affected area or areas in each group were assessed. There was no statistical significant difference in the distribution of wounds between the two groups. ($p>0.05$). The malar, nasal and the temporal regions were found to be the most commonly affected areas in both groups. Abrasions over these regions were primarily superficial while wounds over the lip presented with deeper defects (>2mm).

Pain

Evaluation of pain between the two groups revealed increased mean pain scores in the collagen group as compared to the chitosan group over the first 24 hours (when the patient was on analgesics). A review after one week, when the patients were off all medications, revealed greater mean pain scores in the chitosan group as compared to the collagen group. These differences in both groups (after 24 hours and 1 week), however, were not statistically significant ($p=0.14$ and $p=0.61$).

While there was a reduction in pain at the end of a week in both groups, the drop in scores was found to be statistically significant in the collagen group than in the chitosan group (table 2).

Rate of Wound Healing

Comparing the wound healing rates between the 2 materials, revealed a mean of 9.53 days in chitosan group to that of 10.60 days in the collagen groups. This difference in healing rates were marginally significant ($p=0.05$) favoring chitosan.

Scar Judgement

The Vancouver scale given by Draaijers et al assesses the scar in 4 domains: vascularity, pigmentation, pliability and height (table 3).The scores range from 0 to 13. The maximum score indicates the worst result and 0 score indicates normal skin.

Pigmentation

In the chitosan group, a majority of patients (n=17), showed a return to normal pigmentation, while the remaining healed with some degree of hyper- and hypo-pigmentation. The collagen group, however, revealed a greater percentage of healing with Hypo-pigmenation (46.7%) in the 2 month follow up. Only 33.3% patients revealed a return to normal pigmentation.

This difference in normal pigmentation and hypo/hyper-pigmentation between the two groups was statistically significant

favoring chitosan (figure 4).

Vascularity

The vascular regeneration was evaluated and a majority of the patients showed a return to normal vascularity in both groups. (Chitosan= 70%; Collagen=63.3%; p=0.84). Cases with tissue loss were most often associated with pinkish or a red hue which indicated reduced vascularity/neo-angiogenesis in healing related to these deeper defects.

Pliability

No scars remained in the vast majority of cases. However, the nature of contracture or scarring was Supple (Chitosan= 26.7%, Collagen= 20%) in most of the cases which presented with scar formation. Firm scars and rope like consistency were seen in very few cases in both groups. Collagen showed a greater degree of scarring and contracture as compared to chitosan. However, it should be noted that among the patients, one patient in each group showed tendency to develop hypertrophic scars.

Height

Healing with both dressings usually resulted in a return to normal height and contour in approximately 80% patients in both groups. Contracture and scarring resulted in raised scars measuring between 2-5mm in the collagen group (10%).

Discussion

All patients reporting to a clinic or a hospital with facial wounds present with a variety of concerns ranging from aesthetics to the time required for the wound to heal. While a few wound healing materials (eg: collagen, PRFM) have gained recent popularity, other materials (eg: chitosan) are being introduced into the market and require further studies on humans to justify their use. Before healing can begin hemostasis must occur, application of chitosan and collagen will augment the clotting mechanism by increasing platelet adherence to the endothelial vessel wall, thereby sealing it. This study evaluated the wound healing properties and characteristics of chitosan and collagen wound dressings individually and compared the two over a set of pre-determined criteria and objectives. Chitosan, poly D-glucosamine (GlcN), is a de-acetylated derivative of chitin. Chitosan and its oligomers have found various applications owing to their interesting biological properties. Lysozyme slowly hydrolyzes chitosan membrane and produces chito-oligomers that stimulate correct deposition, assembly and orientation of collagen brils in extracellular matrix components. Histologically, chitosan is known to stimulate the migration of inammatory cells and promote cellular organization [3]. Chitosan has recently gained regulatory approval in the USA for use in bandages and other hemostatic agents. As a semipermeable biological dressing, optimizes conditions for healing by maintaining a sterile wound exudate beneath a dry scab, preventing dehydration and wound contamination. Collagen sheet is prepared from bovine collagenous tissue by treating tissues with a series of chemical and enzymatic procedures. Subsequently, the collagen is chemical cross-linked, packed, and sterilized. Bovine collagen is, chemically, very similar to human collagen. This is crucial, as everything that deviates too much from its own proteins is rejected by the human immune system. For these reasons, collagen sheets are well qualified for use as an effective wound cover [4]. In our study, the pain over the course of a week appeared to be more in the chitosan group than in the collagen group which could be attributed to the adhesive nature of the chitosan membrane and its requirement to be replaced every 2-3 days. The chitosan membrane (Axiostat) used in this study was a non-resorbable variant of the material. Removal of dressings after a couple of days was tedious to the operator and uncomfortable to the patient which could be attributed to the excessive adhesiveness of chitosan. Therefore, chitosan in its resorbable form might be more efficient and less painful to the patient. While there was a reduction in pain over the course of a week in both groups, the drop in scores was found to be statistically significant in the collagen group than in the chitosan group which could possibly be attributed to the properties of collagen which do not require it to be removed once applied as it peels off over the course of time.

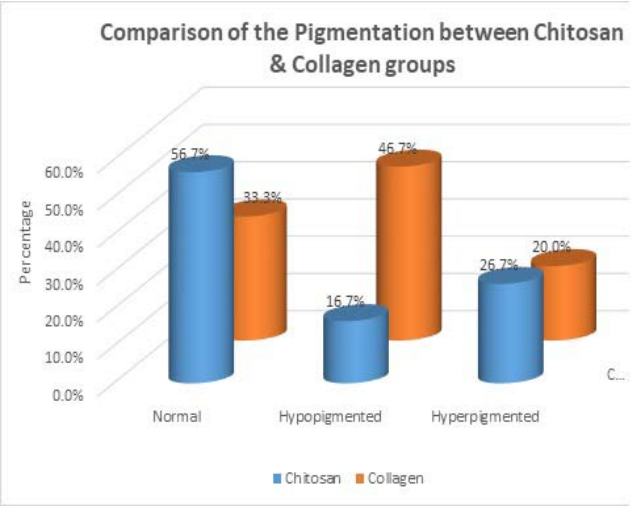

Figure 4: Comparison of the Pigmentation between Chitosan and Collagen Groups

Stone et al. evaluated the healing at skin graft donor sites dressed with chitosan. A total of 20 patients requiring a split-skin graft during the 7-month period were included into the study. The skin graft donor sites were dressed half with chitosan and half with a conventional dressing. Chitosan proved to be an easy dressing material to apply, maintain and was painless to remove which was contradicting to our study [5]. A study conducted by Azad et al, where he evaluated the effects of chitosan versus Bactigras dressing on skin donor sites, revealed that pain sensitivity was less in chitosan group than the Bactigras group [6]. Thoma et al, demonstrated a drop in overall wound sensitivity in wounds in the oral mucosa treated with collagen dressing in comparison to conventional spontaneous healing [7]. It should, however, be noted that the oral cavity provides a constant moist environment which aids in the wound healing process and probably decreases the wound sensitivity. A study was conducted by Munoyath et al., where they compared the wound healing characteristics of amniotic membrane versus collagen on facial abrasions. With regard to pain relief, statistically good results were found in Amnion group when compared to collagen group when the patients were no longer taking analgesics. Pain relieving effect is one of the established properties of amniotic membrane. Its superiority in pain relief in comparison to collagen was attributed to the decreased inflammation, better state of hydration of wound bed and protection of the exposed nerve endings from external irritants [8]. We evaluated and compared the wound healing (via scar judgement) and its rate between chitosan and collagen and observed that healing was marginally faster with chitosan than collagen.

Burkatovskaya et al. compared the antimicrobial ability of HemCom™ bandage, a chitosan acetate bandage, with alginate sponge bandage and silver sulfadiazine cream in mouse models of

infected open wounds. Chitosan acetate was much more effective than other treatments in rapidly reducing bacterial luminescence thus aiding the wound healing process in general [9]. Boynuegri et al. evaluated the effects of chitosan on periodontal regeneration on 20 patients with chronic periodontitis and found that in comparison with the non-treated control group, all treated groups showed statistically significant bone fills when compared with baseline radiographically [10]. This helped improve the rate of periodontal regeneration. In patients undergoing plastic surgery, Biagini et al. treated the donor sites with soft pads of freeze-dried N-carboxybutyl chitosan to promote ordered tissue regeneration. It was found that, compared with control donor sites, better histoarchitectural order, better vascularization and the absence of inflammatory cells were observed at the dermal level, whilst fewer aspects of proliferation of the malpighian layer were reported at the epidermal level. As a result, they concluded that chitosan leads to formation of regularly organized cutaneous tissue and reduces unexpected healing [3,11]. Collagen is a biomaterial that creates a good environment for wound healing by promoting the deposition and organization of freshly formed fibers and granulation tissue in the wound bed [4]. Collagen sheets, when applied to a wound, not only promote angiogenesis, but also enhance body's repair mechanisms by reducing edema and loss of fluids from the wound site. A healthy granulation tissue appeared significantly earlier in amnion treated patients when compared to collagen group in the study conducted by Munoyath et al. In a study conducted by Mohammadi et al, they observed that superficial parts of the burns wound epithelialized faster in patients with amnion dressing [8].

In our study, the healing with both materials were largely comparable and on assessing the resultant scars, significant superiority of chitosan were only observed with regards to pigmentation, while it was on par or marginally better than collagen in the other parameters studied, those being , the vascularity, pliability and height of the scars. Most of the wounds showed degrees of hypo-pigmentation when treated with collagen. Wounds treated with both materials exhibited varying degrees of scarring. Contracture of scars was found to be a more common occurrence with the use of Collagen than with chitosan. In the study conducted by Stone et al., histologically, the skin occluded by the chitosan dressing showed marked differences to skin occluded by the conventional dressing at the newly healed time point. Chitosan biopsies showed a looser connective tissue stroma in the papillary dermis, with better vascular and neural regeneration. In addition, digital color separation analysis of donor site scars demonstrated an earlier return to normal skin color at chitosan-treated areas [3,5]. Another similar clinical study was conducted by Azad et al. to investigate a design for chitosan membrane as a wound-healing dressing. The chitosan membranes, were applied to dress the fresh wound that resulted at a skin graft donor site after removal of the skin layer of 0.010–0.015 inches. Half of the wound was dressed with chitosan membrane and the other half with the control Bactigras. The clinical data indicated that the mesh chitosan membrane promotes efficient adherence, haemostasis, healing and re-epithelialization of the wound [3,6].

Chitosan was also relatively more expensive than collagen. Collagen being a proven material was thus compared with a newer material like chitosan. Collagen is more readily available. Collagen also proved advantageous in the fact that it most commonly required one application after which it shrank and peeled over the duration of healing. Both materials showed relatively poor results when used individually for treating soft tissue injuries with defects than when used with an adjunct such as Platelet Rich Fibrin. The healing and regenerative properties of PRF helped significantly in filling of these defects while using the chitosan or collagen dressings as a barrier membrane. Chitosan produced better margins which merged with the normal adjacent mucosa than collagen which showed raised or everted margins in a few cases. Most of the deeper defects were involving the upper lip region or below the nose and the abrasive wounds were largely confined to the malar and temporal region. The study had its limitations. The lack or deficiency of previous human studies comparing either of the two materials on facial wounds with a significant counterpart, make the discussion of their characteristics difficult.

Conclusions

The results of the present study indicated that the use of chitosan enhanced soft tissue healing and improved color matching between the healing wound sites with the surrounding tissue when compared to collagen. Scarring and contracture of the wounds were more often associated with collagen compared to chitosan. Chitosan (Axiostat), however, required multiple dressing changes. It was, hence, concluded that chitosan can accomplish the requirements of a facial wound dressing material.

References

1. Azuma K, Izumi R, Osaki T, Ifuku S, Morimoto M, Saimoto H, et al. Chitin, Chitosan, and Its Derivatives for Wound Healing: Old and New Materials, Journal of Functional Biomaterials., 6(1), 104-142, 2015.
2. Lionelli GT, Lawrence WT. Wound dressings. Surg Clin North Am. 83(3), 617–638, 2003.
3. Dai T, Tanaka M, Huang Y-Y, Hamblin MR. Expert Rev Anti Infect Ther., 9, 857-879, 2011.
4. Lazovic G, Colic M, Grubor M, Jovanovic M. The application of collagen sheet in open wound healing*. Ann Burns Fire Disasters, 18(3), 151–156, 2005.
5. Stone CA, Wright H, Clarke T, Powell R, Devaraj VS. Healing at skin graft donor sites dressed with chitosan. Br J Plast Surg., 53, 601–606, 2000.
6. Azad AK, Sermsintham N, Chandrkrachang S, Stevens WF. Chitosan membrane as a wound-healing dressing: Characterization and clinical application. J Biomed Mater Res, 69B(2), 216–222, 2004.
7. Thoma DS, Sancho-Puchades M, Ettlin DA, Hämmerle CHF, Jung RE. Impact of a collagen matrix on early healing, aesthetics and patient morbidity in oral mucosal wounds - A randomized study in humans. J Clin Periodontol. 39(2), 157–165, 2012.
8. Munoyath SK, Sathishwaran J, Prasad K. Efficacy of human amniotic membrane and collagen in maxillofacial soft tissue defects - A comparative clinical study. J Oral Maxillofac Surgery, 27(6), 786–790, 2015.
9. Bee MA. NIH Public Access., 75(5), 1781–1791, 2009.
10. Boynuegri D, Özcan G, Senel S, Uç D, Uraz A, Öçgus E, et al. Clinical and radiographic evaluations of chitosan gel in periodontal intraosseous defects: A pilot study. J Biomed Mater Res - Part B Appl Biomater., 90 B(1), 461–466, 2009.
11. Biagini G, Pugnaloni A, Damadei A, Bertani A, Belligolli A, Bicchiega V, et al. Morphological study of the capsular organization around tissue expanders coated with N-carboxybutyl chitosan. Biomaterials., 12(3), 287–291, 1991.