Original Article – Comparative Study

Maxillofacial Soft-tissue Healing Efficacy between Nano-chitosan and Collagen–Chitosan Membrane – A Comparative Study

Harish Kumar N, Soumi Samuel, Suseela Mathew[1], M. Rosemol Jacob[1], P. Amruth[1]

Department of Oral and Maxillofacial Surgery, A B Shetty Memorial Institute of Dental Sciences (ABSMIDS), NITTE University (Deemed to be University), Mangalore, Karnataka, [1]Division of Biochemistry and Nutrition, ICAR-Central Institute of Fisheries Technology, Kochi, Kerala, India

Abstract

Introduction: Routine wound management in maxillofacial trauma with soft-tissue injury needs to be addressed in a systematic way to prevent untoward complications. In this study, we examined the effects of a novel surgical dressing material on pain, wound healing and scar and its feasibility to common people. Our aim is to compare the efficacy and potency of the nano-chitosan membrane and collagen–chitosan membrane as surgical dressing materials for soft-tissue wounds in the maxillofacial region. **Materials and Methods:** Thirty participants who sustained soft-tissue injury in the maxillofacial region were included in the study. Post-suturing, Group A participants were treated with nano-chitosan membrane impregnated with chlorhexidine, Group B participants were treated with collagen–chitosan membrane impregnated with chlorhexidine and Group C participants had received chlorhexidine powder as conventional wound care management and recalled and evaluated for wound healing, pain and scar at seventh day, one month and three months postoperatively. **Results:** The wound healing efficacy of both Group A and B participants was nearly comparable and Group A had better wound healing (P = 0.043) when compared to conventional chlorhexidine dressing material. In relation to pain intensity, Group A was reported with a low intensity of pain and also with better results in scar assessment at the third-month follow-up. **Discussion:** This study had proven that even though the wound healing efficacy of both nano-chitosan and collagen–chitosan membranes is nearly comparable, nano-chitosan shows better results on the evaluation of parameters such as wound healing, pain and scar. Nano-chitosan membrane has better wound healing when compared to conventional chlorhexidine dressing material.

Keywords: Chitosan, chlorhexidine, collagen–chitosan, nano-chitosan, Visual Analogue Scale

Introduction

The soft-tissue wound in the maxillofacial region is treated for enhanced quality of repair. A new dressing material that has maximum advantages over other materials needs to be investigated. The current dictum in soft-tissue management is based on moist wound healing.[1] The main action of chitosan particles in 'chitosan membrane', would be alteration of cell permeability and lysis of the cell membrane as it acts against a negative charge of the cell membrane.[2] The hydrophilic nature of chitosan, a deacetylated derivative of chitin, promotes cellular adhesion and proliferation.[3] This study aims to explore chitosan membrane as an advantageous dressing material.

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Materials and Methods

The present study was approved by the Institutional Ethical Committee (ABSM/EC/80/2021) on January 9, 2021, with participants who had reported to the casualty outpatient

Address for correspondence: Dr. Soumi Samuel, Professor and Postgraduate Guide, A B Shetty Memorial Institute of Dental Sciences, NITTE (Deemed to be University), Deralakatte, Mangalore, Karnataka, India.
E-mail: soumisamuel@gmail.com

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initially and was then referred to the Department of Oral and Maxillofacial Surgery of our college. All procedures performed in the study were conducted in accordance with the ethical standards given in the Declaration of Helsinki. Thirty participants were evaluated and randomly selected based on inclusion and exclusion criteria for the study between January 2021 and December 2022. The procedure and its possible risks and benefits had been thoroughly explained to the participants and their guardians, and their informed consent was obtained before the intervention.

Thirty randomly selected participants were evaluated and divided into three groups (Group A, B and C – 10 in each group) based on inclusion and exclusion criteria for the study. Group A participants received nano-chitosan membrane, impregnated with chlorhexidine as dressing material. Group B participants received collagen–chitosan membrane impregnated with chlorhexidine as surgical dressing material. Group C included participants who received chlorhexidine powder as a surgical dressing material. Case history was recorded.

Participants with maxillofacial soft-tissue wounds which were open, clean/contaminated, and were subjected to trauma with associated maxillofacial soft-tissue wounds which were superficial or deep such as laceration measuring above 2 cm × 1 cm (length × breadth) and who had completed 18 years of age were included in the study. Participants who were not willing to give consent, who had systemic co-morbidities and pregnant women were excluded from the study.

Statistical analysis

With 95% confidence level and 80% power with respect to the research efficacy of human amniotic membrane and collagen in maxillofacial soft tissue defects – A comparative clinical study by Sejal K. Munoyath *et al*., sample size came to be a minimum of 10 in each group.

Evaluation

Wound healing was assessed using 'Wound Evaluation Scale' on post-operative day (POD)-0 and 7.[4] Pain was assessed using the 'Visual Analog Scale' (VAS) on POD 0 and 7.[5] Scar was assessed using the 'Manchester Scar Scale' on first and third month.[6]

Results

The mean wound healing evaluation on the seventh day in Group A, Group B and Group C was 4.3, 4.1 and 2.8, respectively. The difference of mean wound healing between Group A and Group B and that between Group A and Group C were 0.2 each, with $P = 0.038$, which was statistically significant. However, the difference of mean wound healing between Group B and Group C was not found to be statistically significant ($P = 0.079$).

The mean VAS was compared with the three groups using the ANOVA test, wherein the value of Group A was 1.5, and that of Group B was 2.1 and that of Group C was 1.9. Pain was less experienced in Group A.

The mean Manchester scar assessment was done and it was found to be 11.7, 10.9 and 11.6 in Groups A, B and C, respectively. Statistical significance difference was found by using ANOVA (*f* test) and it shows insignificant result. Manchester scar assessment for three months in Group A, Group B and Group C was 7.1, 7.1 and 8.4, respectively. Manchester scar assessment was done by comparing the three groups to find out the difference from first month to third month by applying Fisher's test (ANOVA). The difference in Group A was 4.6 but in Group B was 3.8 having the least difference of 3.2 in Group C. This difference among the three groups was found to be significant ($P = 0.043$). Intercomparison was done and the difference of mean scar assessment between Group A and Group B, Group A and Group C was 0.8 and 1.4, respectively. However, the difference of mean scar assessment between Group B and Group C was 0.6 which is not statistically significant ($P = 0.5$).

Discussion

Among the eminent aspects of oral and maxillofacial surgery, 'trauma' holds an important position in both minor and major surgery and has been managed with both conservative and surgical lines of intervention. The surgical intervention offered for participants with soft-tissue injury like laceration would be of much concern as it affects the aesthetics primarily, along with function.

Chlorhexidine, acts on both extracellular and intracellular membranes of the cell disrupting its integrity. This leads to the leakage of all the cellular contents out into the extracellular environment and brings about the cell death by dehydration and inability to generate Adenosine Tri-Phosphate (ATP) for the survival of the cell. There exists moderate-quality evidence supporting the use of chlorhexidine powder (0.05%) for preoperative skin preparations. The current standard of care for extraoral wounds consists of swabbing for infection, cleaning and dressing. The choice of dressing depends on several factors, such as size, depth, location and type of wound. Participants in all three groups were evaluated for wound healing and pain at POD-0 and POD-7 and scar at an interval of one month and three months.

On comparing mean wound healing on POD-7, with the aid of the 'Wound Evaluation Scale' results were nearly equally comparable for Group-A and Group-B, whereas it is very less in Group-C. Intergroup comparison of wound healing in POD-7 amongst the three groups shows an interesting result. There is a statistically significant difference in wound healing parameters between Group-A and Group-C participants [Table 1 and Figure 1].

A study by Barreras *et al.,*[7] on the use of chlorhexidine and chlorhexidine combined with nano-chitosan in periapical surgeries showed significantly higher bacterial inhibitory activity of the nano-chitosan membrane and thereby improving the healing of soft tissues. This study was in accordance with our study in enumerating the efficacy of the nano-chitosan membrane impregnated with chlorhexidine in wound healing.

However, there was no statistically significant difference in wound healing parameters between Group-A and Group-B and Group-B and Group-C.

The next objective is pain, which is evaluated through 'VAS' and results obtained on POD-7 were statistically not significant in all three groups but comparatively Group-A participants experienced less pain [Table 2 and Figure 2]. There are not many literature support for pain assessment for participants undergoing similar intervention. A study on the application of chitosan-based nanoparticles by Loo *et al.,*[8] is in accordance with favouring the anti-inflammatory property of the material.

The current study had also assessed scar on patient's sutured site at 1 month and 3 month intervals, respectively, using the 'Manchester Scar Scale'. Mean Manchester Scar assessment was done in all three groups and results were proved to be insignificant at 1 month and 3 months, respectively [Table 3 and Figure 3]. An *in vivo* comparative study of wound healing and scar treatment effect on chitosan nanoparticle complex by Nguyen *et al*.,[9] states that chitosan nanoplexes coated with other particles were effective in the preparation of formulation for scar treatment because of its low cost and efficiency.

Even though the present study does not show any statistical significance in scar assessment at one month and three months, the difference of the mean of the Manchester Scar Scale was found to be significant [Table 4 and Figure 4]. In comparison with other dressings (such as creams, gauze, films, sheets, powders and hydrocolloids) hydrogels are biodegradable and biocompatible polymers which have natural origins and could be more effective, useful and play an important role in wound healing [Figure 5]; moreover, they seem like the natural tissues in terms of enzymatic degradation. This study by Khademhosseini and Langer[10] is in accordance with the present study which also explains the wound-healing property of the chitosan membrane.

Barman *et al.,*[11] prepared films made of chitosan nanocomposite loaded with norfloxacin (an antibiotic drug) for sustained release of the drug. This biofilm showed good antimicrobial activity and high biocompatibility and also, it was reported in the literature that the water uptake of the film was limited, which indicates the behaviour of sustained release of the incorporated drug, which supports the present study.

Amiri *et al.,*[12] developed chitosan nanofibres with 4% teicoplanin and demonstrated a stronger antibacterial activity compared with 2% teicoplanin, whereas no significant differences were found between 2% and 4% of the antibiotic solution itself. In a study by Radwan-Pragłowska *et al*.,[13] the nanocomposites are found to be capable of controlled drug release, and transdermal delivery systems were confirmed to be nontoxic to some of the mouse fibroblasts by XTT assay which is used to measure cellular

Table 1: Comparing Mean wound healing evaluation -7th day among the three groups

	Mean	Standard deviation	Minimum	Maximum
Nano-chitosan membrane	4.300	1.252	2.00	6.00
Collagen- chitosan membrane	4.100	1.287	3.00	6.00
Chlorhexidine powder	2.800	1.317	1.00	6.00

F=4.016, *P*=0.03 significance

Table 2: Comparing Mean visual analog scale- 7th day – among the three groups

	Mean	Standard deviation	Minimum	Maximum
Nano-chitosan membrane	1.500	1.269	0.00	4.00
Collagen- chitosan membrane	2.100	1.729	0.00	6.00
Chlorhexidine powder	1.900	1.101	0.00	4.00

F=0.482 *P*=0.623 non-significant

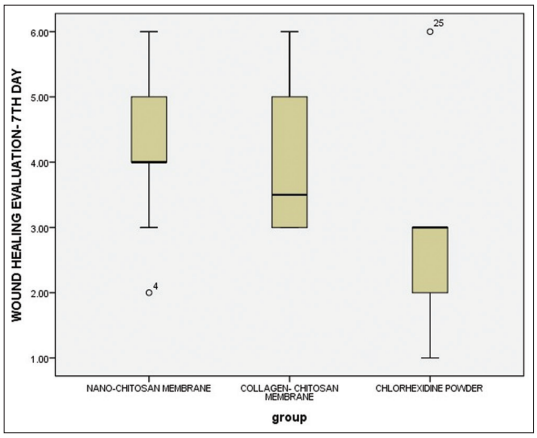

Figure 1: Comparison of mean wound healing evaluation at 7th day of Group A was 4.3 and that of Group B and Group C was 4.1 and 2.8 respectively. [o25 - It is the box plot representation representing the extreme values of the given date, 25th data is extreme]

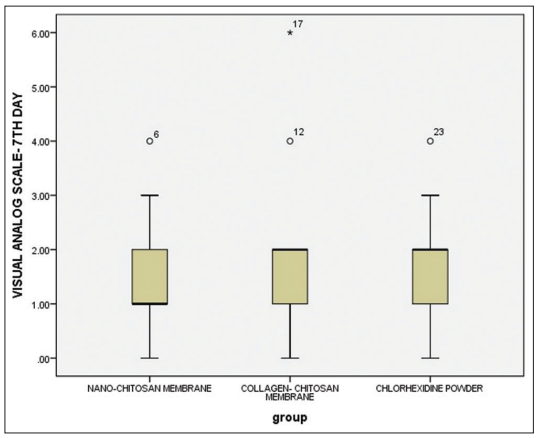

Figure 2: Comparison of mean visual analog scale evaluation with the three groups in postoperative day-7 was found to be 1.5 in Group A, 2.1 in Group B which was maximum and 1.9 in Group C [*17 - It is the box plot representation representing the extreme values of the given date, 17th data is extreme]

metabolic activity as an indicator of cell viability, proliferation and cytotoxicity, which supports the present study.

Chitosan interferes with bacterial metabolism by electrostatic stacking at the surface of the bacteria. It also blocks the transcription of RNA by intercalation of chitosan on DNA chains. It has a high renal clearance and undergoes acid-catalysed degradation. It is also susceptible to enzymatic degradation by lysozyme. This property of chitosan is explained in the literature by Del Prado-Audelo *et al.,*[14] which is in accordance with the present study.

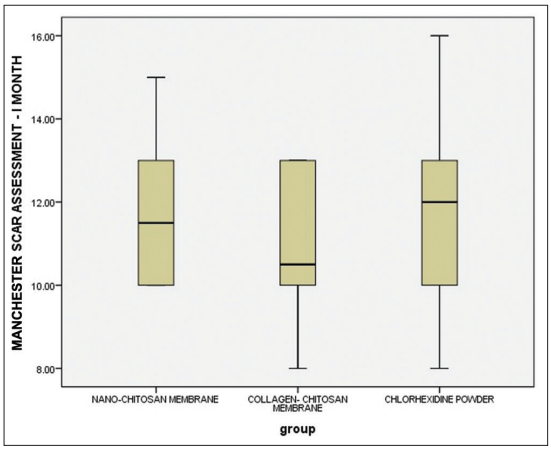

Figure 3: Comparison of mean Manchester scar scale shows assessment of scar done at 1 month for all three groups. It was found to be 11.7 in Group A and 10.9 in Group B and it was 11.6 in the Group C

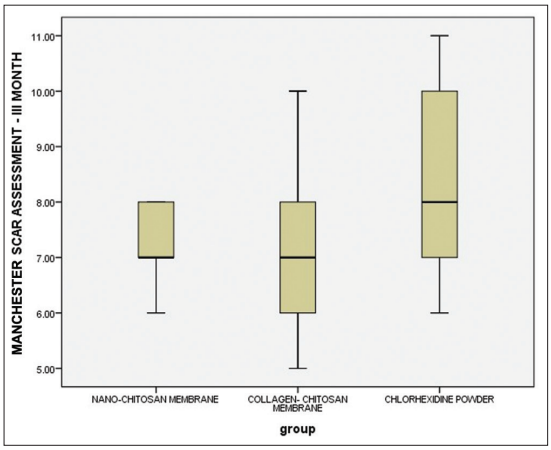

Figure 4: Mean Manchester scar scale - first month and third month shows Manchester scar assessment for first month and third month. The difference in Group A was 4.6 but in Group B, it was 3.8 having the least difference of 3.2 in Group C

Mahdavinia *et al.,*[15] used the ciprofloxacin-loaded nanocomposite hydrogels and showed its antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria, which supports the present study of its antimicrobial activity. The collagen membrane, have gained importance in various clinical fields, especially in wound healing.[16]

In the present study, we encountered two participants (one each in Group-A and Group-B) who had taken analgesic medications for more than the prescribed period since they had other complaints of the body such as fractures of long bones such as femur or humerus. However, none of the participants in our study reported with any possible kind of allergies in any form or discomfort. We considered patient education as an imperative part of treatment. Therefore, we had counselled and motivated all the participants who had willingly given consent for the study and continued our follow-up at the correct interval by intimating the patients through telecommunication.

The results of this study threw light on the significance of chitosan membrane in oral and maxillofacial surgery and its application in wound management. Even though it was evident that chitosan membrane had significantly proven to be effective in the healing of wounds and minimalising scar postoperatively, further research had to be encouraged to establish the potency of chitosan membrane in the evaluation of wound healing, pain and scar in maxillofacial soft-tissue wounds.

The limitations of our study were that, since it was a single-centre study, the results cannot be generalised to a large

Table 3: Comparing Mean Manchester scar assessment - I month among the three groups

	Mean	Standard deviation	Minimum	Maximum
Nano-chitosan membrane	11.700	1.252	2.000	6.000
Collagen- chitosan membrane	10.900	1.792	8.000	13.000
Chlorhexidine powder	11.600	2.271	8.000	16.000

F=0.516 *P*=0.603 non-significant

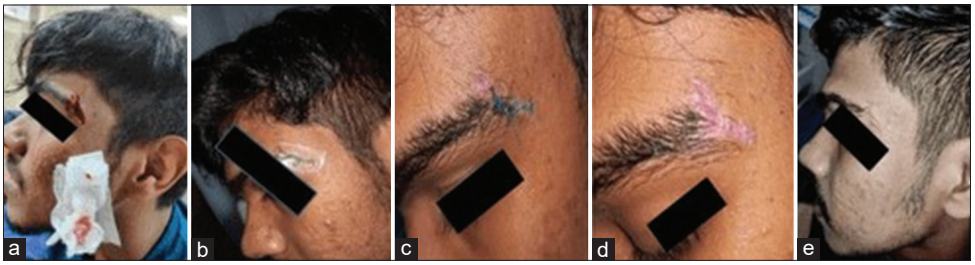

Figure 5: Patient photograph with follow-up (pre and post intervention). (a) Soft-tissue injury before suturing, (b) Postsuturing and placement of the membrane, (c) Postoperative seventh day, (d) Postoperative first month, (e) Postoperative third month

Table 4: Difference of Mean manchester scar assessment - I month - III month

	Mean difference	Standard deviation	Minimum	Maximum
Nano-chitosan membrane	4.600	1.265	3.00	7.00
Collagen- chitosan membrane	3.800	1.033	2.00	5.00
Chlorhexidine powder	3.200	1.229	1.00	5.00

F=3.543 *P*=0.043 significant

population, which might warrant the need for future studies with a wider population. Inappropriate usage of analgesic medication by two patients for addressing other concerns of the body had been reported during the interventional period.

Conclusion

Nano-chitosan membrane has better wound healing when compared to conventional chlorhexidine dressing material. The wound healing efficacy of both nano-chitosan and collagen–chitosan membranes is nearly comparable. Nano-chitosan membrane, collagen–chitosan membrane and chlorhexidine dressing material have no significant effect on pain, but nano-chitosan membrane dressing proved to be having less pain comparatively. Scar assessment also proves to be insignificant among all three groups in first month and third-month follow-up. When compared to the quality of scar from first month to third month (difference of mean of scar assessment), our study shows statistical significance. The use of nano-chitosan membrane incorporated with chlorhexidine can be used as an alternative dressing material for all participants, targeting especially for participants with financial constraints.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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