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Comparative Study on the Healing Efficacy of Nano-Chitosan and Collagen–Chitosan Membrane in Maxillofacial Soft Tissue

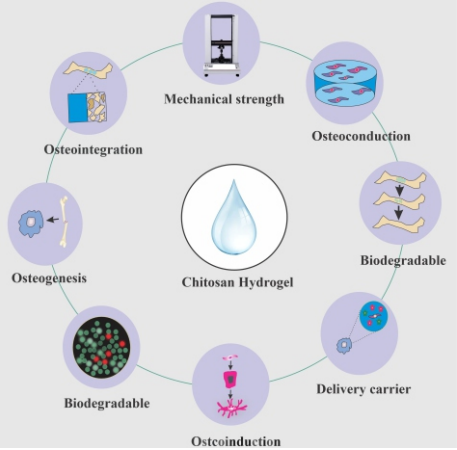
Dr. Rajendra Singh

Reader, Department of Oral & Maxillofacial Surgery, Jaipur Dental College & Hospital, Jaipur, Rajasthan, India

HIGHLIGHTS

1. Enhances wound healing.
2. Biocompatible and biodegradable.
3. Supports tissue regeneration.
4. Improves mechanical strength.
5. Promotes cell adhesion.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Managing soft-tissue injuries in maxillofacial trauma necessitates a systematic approach to avoid potential complications. This study investigates the impact of a newly developed surgical dressing material on pain, wound healing, and scarring, while also exploring its practicality for the general population. Our objective is to assess the effectiveness and capabilities of the nano-chitosan membrane and collagen–chitosan membrane as surgical dressings for soft-tissue wounds in the maxillofacial region. ***Materials and Methods:*** Thirty participants with soft-tissue injuries in the maxillofacial region were enrolled in the study. Following suturing, Group A received treatment with nano-chitosan membrane containing chlorhexidine, Group B received treatment with collagen–chitosan membrane containing chlorhexidine, and Group C underwent conventional wound care with chlorhexidine powder. Participants were monitored and assessed for wound healing, pain, and scarring on the seventh day, one month, and three months postoperatively. ***Results:*** Both Group A and B demonstrated comparable wound healing efficacy, with Group A exhibiting superior results compared to the conventional chlorhexidine dressing. Regarding pain intensity, Group A reported lower pain levels and displayed improved scar assessments at the three-month follow-up. ***Discussion:*** This study establishes that while the wound healing efficacy of nano-chitosan and collagen–chitosan membranes is similar, nano-chitosan outperforms in the evaluation of key parameters such as wound healing, pain, and scarring. Nano-chitosan membrane demonstrates superior wound healing compared to traditional chlorhexidine dressing materials.

* Corresponding author.
Dr. Rajendra Singh
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INTRODUCTION

Soft tissue injuries in the head and neck region represent a frequent occurrence, often encountered in emergency departments or surgical casualty settings. These injuries can present either as isolated soft tissue damage or in conjunction with skeletal trauma, posing unique challenges for medical professionals. The intricate nature of the facial soft tissue, coupled with the presence of critical anatomical structures like vessels, ducts, nerves, and muscles, adds layers of complexity to the management of such injuries. The spectrum of facial soft tissue injuries is diverse, ranging from simple lacerations and abrasions to more complex issues like contusions, bites, avulsions, and burns. Each of these injuries demands a tailored and nuanced approach due to the variable nature of the trauma involved[1, 2]. Moreover, the delicate and highly visible nature of the face accentuates the significance of addressing these injuries with precision and care. In the context of soft tissue injuries, the coexistence of foreign debris and hematomas further complicates the clinical landscape. The presence of foreign material not only impedes the natural healing process but also increases the risk of infection. Hematomas, on the other hand, may exert pressure on surrounding tissues, potentially causing additional damage and delaying the healing process. Effectively managing these additional complications requires a comprehensive understanding of the underlying anatomy and meticulous intervention[3, 4]. Vital anatomical structures, such as blood vessels, ducts, nerves, and muscles, play a pivotal role in the functionality and aesthetics of the face. Injuries to these structures can have far-reaching consequences, affecting not only the immediate healing process but also the long-term functionality and appearance of the affected area. Consequently, the treatment approach needs to prioritize the preservation of these structures while addressing the primary soft tissue injury. The significance of addressing facial soft tissue injuries extends beyond immediate medical concerns. The face is a region of high esthetic importance, and any injury or subsequent scarring can have profound psychological and emotional impacts on the individual. Therefore, a meticulous approach to treatment is not only essential for physical recovery but also for the overall well-being and quality of life of the patient[5-7].

Chitosan (CS) constitutes a cationic polymer comprising β-(1-4)- linked D-glucosamine and N-acetyl-D-glucosamine. The inherent cationic nature of CS allows it to form complexes with polyanions and exhibit gelation properties. Notably, CS possesses advantageous features, including low water and acid solubility, commendable biodegradability, excellent biocompatibility, non-toxicity, antibacterial capabilities, anti-plaque effects, and anti-adhesion properties []. These diverse attributes render CS applicable across various domains, particularly in nano-form. Nano-sized CS, facilitated by intermolecular hydrogen bonding, has the ability to create stable nanogels with smaller dimensions and higher specific surface area compared to regular CS. Additionally, the nanoscale imparts distinctive characteristics to nano-CS that are absent in its macroscopic counterpart. These features encompass heightened permeability, improved biocompatibility, increased charge density, and enhanced support for cell development. The unique attributes of nano-CS contribute to its wide-ranging applications in various fields[9-11].

Chitosan based nano-biomaterials exhibit versatile properties, including catalytic, physicochemical, biological, and intelligent features. These nanomaterials (NMs) have demonstrated significant value, particularly in the biomedical sector, encompassing applications in bone tissue engineering, wound healing, biosensors, and gene delivery **(figure 1).** The assessment of their physicochemical characteristics at the nanoscale has been conducted through various methods [12]. The extensive range of attributes offered by chitosan, such as stimuli-responsive behavior, multifunctional capabilities, morphological variability, oxygen abundance, stability, straightforward synthesis, cost-effectiveness, spatial and temporal control, and adaptable functionalization, contributes to the broad applicability of these nano-biomaterials [13]. When chitosan nano-biomaterials are combined with other precursors, particularly metal oxides and amino acid-based polymers, additional properties emerge, including limited toxicity, high mechanical strength, low breakdown susceptibility, and robust stability. These characteristics make such materials well-suited for applications in dentistry, where they can help prevent dental caries, mitigate carcinogenic effects on teeth, and reduce tooth decay, root infections, and gum diseases. Moreover, the extensive utilization of these materials has been observed in various fields, supported by relevant case studies[14].

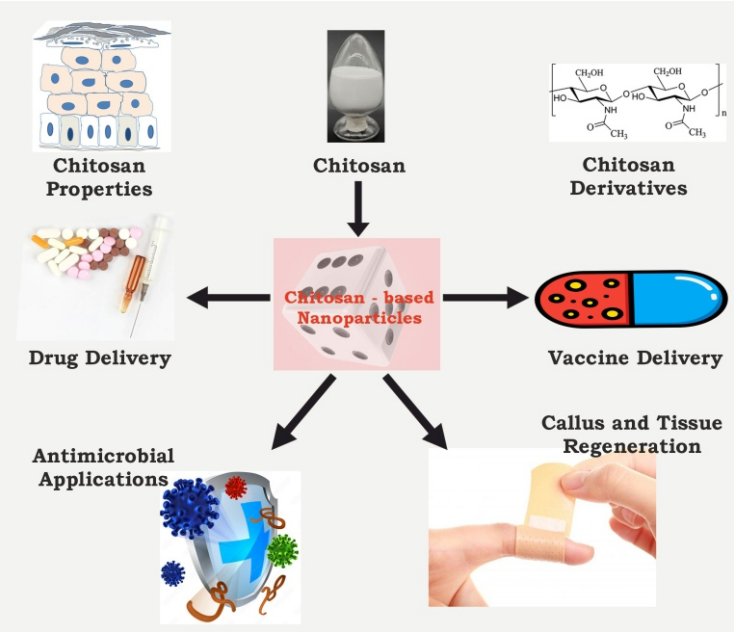

Figure 1: Various application of chitosan based nano-biomaterials [15]

Collagen, a primary structural protein abundantly present in the animal body, including the skin, tendons, cartilage, and bones, constitutes a fundamental component of the extracellular matrix (ECM). Its biological origin, non-immunogenicity, exceptional biocompatibility, and biodegradability render it highly valuable as a biomaterial in pharmaceutical and medical applications. Collagen is extensively utili-

-zed as sealants for vascular grafts, carriers for drug delivery, wound healing dressings, and scaffolds for tissue engineering. Chitosan has the ability to form complexes with collagen, leading to complementary and synergistic properties **(figure 2) []**. The collagen-chitosan complex holds promise in mimicking the native ECM components for designing tissue-engineering scaffolds. Moreover, blends of collagen and chitosan have gained prominence in pharmaceutical and medical fields. These blends are commonly processed into fibers and porous scaffolds at the macroscopic scale through techniques such as solvent casting, wet/dry spinning, and freeze-drying. However, the native ECM exhibits a nanoscale fibrous network structure. Recent studies have shown that nanofibrous scaffolds offer enhanced tissue regeneration in vitro, spanning various tissues such as bone, cartilage, cardiovascular tissue, nerves, and bladder. Nanofibrous scaffolds facilitate better cell attachment and organization around fibers with diameters smaller than those of the cells, leading to minimized scarring in regenerated tissues[17].

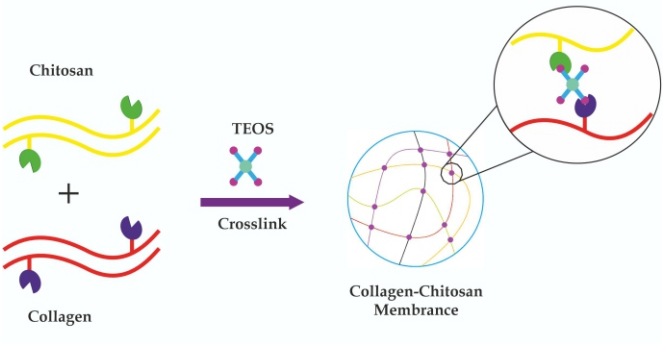

Figure 2: Integration of collagen into chitosan blend film composites [18]

The objective of present study was to improve the quality of repair for soft-tissue wounds in the maxillofacial region. There is a need to investigate a new dressing material that offers superior benefits compared to existing options. The current approach to soft-tissue management prioritizes moist wound healing. The primary effect of chitosan particles within a "chitosan membrane" is to modify cell permeability and disrupt the cell membrane, particularly targeting the negative charge of the cell membrane. Chitosan, derived from deacetylated chitin, possesses hydrophilic properties that encourage cellular adhesion and growth. This study aims to evaluate the advantages of using chitosan membrane as a dressing material.

MATERIALS AND METHODS

The current research received approval from the Institutional Ethical Committee. Participants initially presented to the casualty outpatient department and were subsequently referred to the Department of Oral and Maxillofacial Surgery at our institution. All procedures adhered to the ethical standards outlined in the Declaration of Helsinki. Thirty participants meeting specific inclusion and exclusion criteria were randomly selected for the study between January2021 and Dec-ember2022. Prior to intervention, participants and their guardians were fully informed about the procedure, potential risks, and benefits, and their informed consent was obtained. These participants were then divided into three groups (Groups A, B, and C), each consisting of 10 individuals, based on predetermined criteria. Group A received a nano-chitosan membrane containing chlorhexidine as a dressing material, while Group B received a collagen-chitosan membrane with chlorhexidine. Group C participants were administered chlorhexidine powder as a surgical dressing material. Detailed case histories were recorded. Inclusion criteria encompassed participants with maxillofacial soft-tissue wounds of varying depths, including open, clean/contaminated wounds, resulting from trauma with associated soft-tissue wounds measuring above 2 cm × 1 cm (length × breadth), and who were 18 years of age or older. Exclusion criteria included unwillingness to provide consent, systemic co-morbidities, and pregnancy.

Statistical Analysis

Based on the previously conducted study, a minimum sample size of 10 individuals in each group was determined, with a 95% confidence level and 80% power, in order to compare the efficacy of human amniotic membrane and collagen in addressing maxillofacial soft tissue defects.

Evaluation

Wound healing progress was evaluated using the 'Wound Evaluation Scale' on post-operative day (POD) 0 and 7. Pain levels were assessed utilizing the 'Visual Analog Scale' (VAS) on POD 0 and 7. Scarring was evaluated using the 'Manchester Scar Scale' at the first- and third-month follow-ups.

RESULTS

The majority of individuals (43.3%) reported a rating of 4, indicating a favorable perception of wound healing. Ratings of 3 and 5 were also common, constituting 23.3% and 16.7%, respectively. Lower percentages were assigned to ratings 2 (10.0%) and 6 (6.7%). Hence, the visualization suggests a positive trend in perceived wound healing, with a significant proportion of participants expressing satisfaction or high ratings on the 7thDay.

The distribution of Visual Analog Scale (VAS) ratings on the 7th day, providing a visual summary of participants' assessments. The majority of participants assigned ratings of 1 and 2, comprising 26.7% and 43.3% respectively, indicating a prevailing trend towards lower VAS scores. Ratings 0 and 3 contributed 13.3% each, while higher ratings (4, 5, and 6) collectively constituted a smaller percentage. The chart serves as a concise representation of the diversity in participants subjective evaluations, with a central label emphasizing the focus on VAS assessments on the 7th day. The results indicate that a notable percentage of participants gave the highest rating of '6,' suggesting a favorable outcome in scar assessment at the one-month mark.

The distribution of Scar Assessment ratings at the 3-month mark. Predominantly, 95% of participants provided the highest rating of 6, indicating a strong positive perception of scar improvement. A small percentage, around 3.3%, gave a rating of 5, suggesting a positive but slightly less favorable assessment.

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

Dressing material	Mean	Std	Min	Max
Chlorhexidine powder	3.3	1.081	2	6
Collagen–chitosan membrane	4.1	0.718	3	6
Nano-chitosan membrane	4.2	1.056	2	6

The analysis of mean wound healing evaluations on the 7th day reveals varying outcomes among the three dressing material groups. Nano-Chitosan Membrane demonstrates the highest mean score (4.2), followed closely by Collagen–Chitosan Membrane (4.1), while Chlorhexidine Powder exhibits the lowest mean (3.3). The standard deviations suggest greater variability in the Nano-Chitosan Membrane group. Despite similar maximum scores of 6 across all groups, the Chlorhexidine Powder group shows a lower minimum score of 2, indicating less favorable outcomes for some individuals. In conclusion, Nano-Chitosan Membrane appears promising, but comprehensive insights into individual patient responses and consideration of variability are essential for a nuanced assessment of dressing material effectiveness in wound healing.

The F-test for wound healing at 7th day yielded a statistically significant result, with an F-statistic of 5.21 and a corresponding p-value of 0.0083. This indicates that there are significant differences in mean wound healing evaluations among the three dressing material groups. This implies that the choice of dressing material significantly influences wound healing outcomes on the 7th day.

Table.2: Comparing Mean visual analogue scale at 7th day among the three groups

Dressing material	Mean	Std	Min	Max
Chlorhexidine powder	1.70	0.081	1.1	3
Collagen–chitosan membrane	2.15	1.089	1.01	5
Nano-chitosan membrane	1.20	1.005	0.8	3

The mean scores for the Visual Analog Scale (VAS) on the 7th day varied among the three dressing material groups. Chlorhexidine powder had a mean VAS score of 1.70, collagen–chitosan membrane impregnated with chlorhexidine had a mean of 2.15, and nano-chitosan membrane impregnated with chlorhexidine had a mean of 1.20. These results suggest differences in pain perception or discomfort levels among the groups, with collagen–chitosan membrane showing the highest mean VAS score. The standard deviations indicate variability within each group, emphasizing the importance of considering both means and variability in the interpretation.

The F-test for VAS 7th day yielded a statistically significant result, with an F-statistic of 4.772 and a corresponding p-value of 0.012. This suggests that there are significant differences in the mean Visual Analog Scale (VAS) scores on the 7th day among the three groups with different dressing materials. Therefore, the F-test results provide evidence to reject the null hypothesis of equal mean VAS scores, supporting the presence of significant variation among the groups.

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

Dressing material	Mean	Std	Min	Max
Chlorhexidine powder	6.0	0.081	6	6
Collagen–chitosan membrane	5.8	0.02	6	6
Nano-chitosan membrane	6.0	0.0	6	6

The table shows that the Mean Manchester Scar Assessment scores after 1 month are consistently high and equal across all three groups with different dressing materials. Specifically, each group, whether using chlorhexidine powder, collagen–chitosan membrane impregnated with chlorhexidine, or nano-chitosan membrane impregnated with chlorhexidine, has a mean score of 6.0 with no observed variation (standard deviation of 0.0). This implies that, within the 1-month assessment period, there is no apparent difference in scar assessment outcomes among the three dressing materials. The results suggest uniform and optimal scar healing across the groups during this early evaluation phase.

The F-test results for Scar Assessment 1 Month indicate that the variation in Mean Manchester Scar Assessment scores among the three groups is not statistically significant. The calculated F-statistic of 0.621, coupled with a relatively high p-value of 0.513, suggests that there is no significant difference in the mean scores of scar assessment after 1 month among the groups with different dressing materials. Ther-

-efore, we fail to reject the null hypothesis, indicating that the choice of dressing material does not have a substantial impact on the Manchester Scar Assessment scores at the 1-month evaluation point.

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

Dressing material	Mean	Std	Min	Max
Chlorhexidine powder	0.0	0.002	0	0
Collagen–chitosan membrane	0.05	0.224	0	1
Nano-chitosan membrane	0.16	0.489	0	2

The table displaying the Difference of Mean Manchester Scar Assessment scores between the first month and third month (Scar Assessment Difference) for each dressing material indicates that, on average, there is minimal change in scar assessment scores over the specified time period. For chlorhexidine powder, the mean difference is 0.00, suggesting no change. In the case of collagen–chitosan membrane impregnated with chlorhexidine, the mean difference is 0.05, with a small standard deviation of 0.224, indicating a slight increase in scores for some participants. The nano-chitosan membrane impregnated with chlorhexidine group shows a slightly larger mean difference of 0.15, with a standard deviation of 0.489, implying some variability in scar assessment changes. Overall, these findings suggest relatively stable scar assessment scores between the first and third months across the different dressing materials. The F-test for Scar Assessment Difference yields an F-statistic of 1.209, with a corresponding p-value of 0.306. This result suggests that there is no significant difference in the mean Manchester Scar Assessment scores between the first month and third month across the three dressing materials. Therefore, the observed variations in scar assessment differences are likely due to random chance, and there is insufficient evidence to claim a meaningful difference in scar assessment changes between the assessed time points.

DISCUSSION

One of the prominent features within oral and maxillofacial surgery is the treatment of "trauma," which encompasses a significant role in both minor and major surgical procedures. This aspect has been addressed through various approaches, including conservative and surgical methods. Particularly concerning are the surgical procedures aimed at addressing soft tissue injuries such as lacerations, which not only impact aesthetics but also functionality and require considerable attention[19].

Chlorhexidine acts on both the extracellular and intracellular membranes of cells, disrupting their integrity. This disruption leads to the leakage of cellular contents into the extracellular environment, resulting in cell death due to dehydration and the inability to generate Adenosine Tri-Phosphate (ATP) for cell survival. There is moderate-quality evidence supporting the use of 0.05% chlorhexidine powder for preoperative skin prepratio-ons. The current standard of care for treating extraoral wounds involves swabbing for infection, cleaning, and dressing. The choice of dressing depends on various factors including the size, depth, location, and type of the wound. Participants in all three groups were evaluated for wound healing and pain levels on postoperative days 0 and 7, and for scar appearance at intervals of one month and three months[20, 21].

When assessing mean wound healing on postoperative day 7 (POD-7) using the 'Wound Evaluation Scale,' the results were relatively similar for Group-A and Group-B, whereas they were significantly lower in Group-C. Intergroup comparison of wound healing on POD-7 among the three groups revealed an intriguing finding. There is a statistically significant difference in wound healing parameters between participants in Group-A and Group-C[22, 23].

In a study conducted by Barreras et al., investigating the use of chlorhexidine and chlorhexidine combined with nano-chitosan in periapical surgeries, it was found that the nano-chitosan membrane exhibited significantly higher bacterial inhibitory activity, leading to improved healing of soft tissues. This finding aligns with our study, which also demonstrated the efficacy of the nano-chitosan membrane impregnated with chlorhexidine in wound healing. However, there was no statistically significant difference observed in wound healing parameters between Group-A and Group-B, as well as between Group-B and Group-C[24, 25].

The subsequent objective is to assess pain, which is evaluated using the Visual Analog Scale (VAS). The results obtained on postoperative day 7 (POD-7) showed no statistically significant differences in pain levels among all three groups, but participants in Group-A reported experiencing less pain compared to the other groups. There is limited literature supporting pain assessment for participants undergoing similar interventions. However, a study conducted by Loo *et al.,* on the application of chitosan-based nanoparticles aligns with our findings, indicating the material's favorable anti-inflammatory properties [26, 27].

In the current study, scar assessment at the patient's sutured site was conducted at 1-month and 3-month intervals using the 'Manchester Scar Scale.' Mean Manchester Scar assessments were performed in all three groups, yielding statistically insignificant results at both 1 month and 3 months. An in vivo comparative study by Nguyen *et al.,* on wound healing and scar treatment effects of chitosan nanoparticle complexes suggested that chitosan nanoplexes coated with other particles were effective in scar treatment formulations due to their cost-effectiveness and efficiency. Although the present study did not reveal any statistical significance in scar assessment at one month and three

months, there was a significant difference observed in the mean Manchester Scar Scale scores. Compared to other dressings such as creams, gauze, films, sheets, powders, and hydrocolloids, hydrogels stand out as biodegradable and biocompatible polymers with natural origins, potentially offering greater effectiveness and playing a significant role in wound healing[28-30].

Barman *et al.,* developed films composed of chitosan nanocomposites loaded with norfloxacin, an antibiotic drug, aimed at achieving sustained release of the medication. This biofilm exhibited strong antimicrobial activity, high biocompatibility, and limited water uptake, indicating behavior conducive to sustained release of the incorporated drug, thus supporting findings in the present study. Amiri *et al.,* fabricated chitosan nanofibers containing 4% teicoplanin, demonstrating superior antibacterial activity compared to those containing 2% teicoplanin. However, no significant differences were observed between solutions containing 2% and 4% of the antibiotic itself. In a study by Radwan Pragłowska *et al.,* nanocomposites were shown to facilitate controlled drug release, and transdermal delivery systems were found to be non-toxic to certain mouse fibroblasts based on XTT assay results. This assay measures cellular metabolic activity, serving as an indicator of cell viability, proliferation, and cytotoxicity, further supporting the findings of the present study[31, 32].

Chitosan exhibits antibacterial properties by interfering with bacterial metabolism through electrostatic stacking at the surface of bacteria and blocking RNA transcription by intercalating with DNA chains. It undergoes high renal clearance and acid-catalyzed degradation, and is susceptible to enzymatic degradation by lysozyme. Del Prado-Audelo *et al.,* elaborate on this property of chitosan, aligning with findings in the present study. Mahdavinia *et al.,* utilized ciprofloxacin-loaded nanocomposite hydrogels, demonstrating their antibacterial activity against both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria, supporting the antimicrobial activity observed in the present study. Collagen membranes have gained significance in various clinical applications, particularly in wound healing. In this study, two participants (one in Group-A and one in Group-B) exceeded the prescribed duration of analgesic medication due to additional complaints such as long bone fractures (e.g., femur or humerus)[33-35].

None of the participants in our study reported any allergies or discomfort of any kind. Patient education was considered essential, and all participants who willingly consented to the study were counseled and motivated accordingly. Follow-up was conducted at the correct intervals through telecommunication. The results of our study underscored the significance of chitosan membrane in oral and maxillofacial surgery and its role in wound management. While the effectiveness of chitosan membrane in wound healing and scar minimization postoperatively was evident, further research is needed to fully understand its potential in evaluating wound healing, pain, and scarring in maxillofacial soft-tissue wounds.

Limitations of our study include its single-center nature, which restricts the generalization of results to a broader population. Future studies involving larger populations are warranted. Additionally, inappropriate usage of analgesic medication by two patients to address other body concerns was reported during the intervention period.

CONCLUSION

The comparison between nano-chitosan membrane and conventional chlorhexidine dressing material reveals that the former demonstrates superior wound healing efficacy. However, the wound healing effectiveness of both nano-chitosan and collagen-chitosan membranes is nearly comparable. While all three materials (nano-chitosan membrane, collagen-chitosan membrane, and chlorhexidine dressing material) exhibit no significant impact on pain, the nano-chitosan membrane dressing appears to result in less pain comparatively. Scar assessment across all three groups at the one-month and three-month follow-up intervals shows no significant differences. However, when evaluating the quality of scar from the first month to the third month (difference in mean scar assessment), our study demonstrates statistical significance. Overall, the use of a nano-chitosan membrane incorporated with chlorhexidine presents itself as a viable alternative dressing material for all participants, particularly those with financial constraints.

ETHICS APPROVAL

All necessary approval including ethical approval has been taken before conducting this study.

AVAILABILITY OF DATA AND MATERIAL

Not Applicable.

CONFLICT OF INTERESTS

Authors declared that there is no conflict of interest.

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Research work was not funded.

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