AMERICAN JOURNAL OF BIOMEDICINE AND PHARMACY
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Article

Comparison Study the Efficacy of Bacterial- Derived Dna Nanostructure and Nano Chitosan on Cutaneous Wound Healing in Rabbits

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Citation: Farman, R. H. Comparison Study the Efficacy of Bacterial- Derived Dna Nanostructure and Nano Chitosan on Cutaneous Wound Healing in Rabbits. American Journal of Biomedicine and Pharmacy 2026, 3(8), 36-43.

Received: 5th Jul 2026
Revised: 21th Jul 2026
Accepted: 14th Aug 2026
Published: 5th Sep 2026

Abstract: Wounds are amid the most common surgical defect, caused by road accidents and various injuries. In veterinary fields, Fast healing offerings a significant challenge because delayed healing can increase the wound contamination and infection, in addition to the possibility of complications affecting the tissues and organs beneath the injury site. . Therefore, it has become essential to search for new materials and treatment methods that can accelerate wound healing and improve the quality of regenerated tissue . This has led to increased interest in using biomaterials, nanoparticles, and preparations with antimicrobial and tissue-regenerative activity as promising strategies for improving wound treatment outcomes. 60 Newzeland rabbits were concluded in this study which divided randomly in four groups of 15 rabbits , G1:regarded control group , G2: treated Nano DNA with, G3 : Nano chitosan and G4: treated with combination of Nano DNA and Nano chitosan. Complete wounds 2 Cm in diameter Were done, Biopsies Were collected at 7, 14, and 21 days for histopathological evaluation, The healing outcomes were relatively that all treated groups enhanced wound healing compared with the control group after 7 days. While at the 14 days after treatment the 3rd and 4th groups showed noticeable healing processes than other groups, while at 21 days from treatment the animals treated with a combination of Nano DNA and Nano chitosan , almost complete healing considered by occurrence of thick keratinized layer and profuse collagen in the dermis as well as new formation of hair follicle when compared with other groups

Keywords: Wound healing, DNA nanostructures, nano-chitosan, nanomaterials, tissue regeneration, rabbits, histopathology

Introduction

Wound healing is a sequence of cellular and molecular events, begin by inflammation stage , formation of granulation tissue, angiogenesis, collagen deposition, then remodeling. These stages need sufficient energy and regenerative capacity of the tissues to thorough e repair process [1].

So diabetic patient with high glucose levels in blood with low energy in tissue casing foul this procedure either chronic wound infection related with microbial biofilm causing delayed wound healing This establishes an urgent need to find modification to reduce the prevalence of infection [2].

Nano particles

Nano particles acting by two ways, wound dressings, scaffolds or delivery vehicles and specific targeting abilities due to their antibacterial and pro-wound-healing properties silver NPs. These NPs represent a paradigm shift in wound healing therapies [3].

The nanomaterials have been shown to associate in all stages of wound healing including inflammation, proliferation and remodeling .Natural compounds proven antioxidant, anti-inflammatory, immunomodulatory, or antimicrobial characteristics to accelerate the wound healing. wide range of potential uses in treating both acute and chronic wounds [4].

Recent research shows their potential of Nano material in tissue regeneration, enhance therapy accuracy, sustained drug release, and address issues related, especially hydrogels, give significant capacity to transform the future of wound care and may be unified for developing active wound dressing to treat microbial infections based on endogenous triggers, such as pH, temperature, enzymes, and toxins secreted by the bacteria [5].

DNA Nanoparticles

Framework DNA (FNA) is best drug transfer system due to excellent stability and remarkable transport ability. Chronic wound controlling faces three main challenges: infection, inflammatory environments, and decreased regeneration. Bacterial toxins and inflammation delay tissue regeneration and wound reconstruction, causing abnormalities in cell proliferation, migration.so (FNA) nanostructures, developed through DNA nanotechnology find to fix this issue. Either triboelectric-responsive DNA hydrogels used as dressings for treatment of chronic diabetic wounds [6].

DNA stimulates formation and re-epithelialization of extracellular matrix with changes in the communication between macrophages, fibroblasts, and keratinocytes, then simplifying wound healing on the skin [7].

Nano Chitosan

Chitosan is biopolymer polysaccharide modified by partial deacetylation of chitin that find in exoskeletons of shrimps and crustaceans. It is non-toxic polymer with great potential for chemical and mechanical to create new possessions in biomedicine. Including drug management, gene delivery and diagnosis of diseases [8].

Nano Chitosan have greater chemical and physical characteristics such as large surface area, permeability, tensile strength and improved mechanical properties compared to pure chitosan. Because of their characteristics, Chitosan is a suitable for creating extracellular tissue matrixes in tissue engineering. It may be used as a vehicle for the transport of external antimicrobial agents including proteins, genes, and medications used to treat illnesses [9]. Either act as scaffold to keep wound moist and protect from infection. accelerated the wound healing by reducing the inflammation without any scar formation. Create chitosan compounds. Such modified chitosan is widely used in wound healing and several tissue engineering lines [10].

Chitosan have many properties beneficial to wound-healing, such as biodegradability, structural integrity, hydrophilicity, adhesiveness to tissue, and. by promotes fiber proliferation and granulation tissue, angiogenesis (neovascularization) [11].

The antimicrobial activity of chitosan Nanomaterials against bacteria, specially Gram-negative bacteria were more sensitive to the bactericidal action than Gram-positive ones. For medical applications, chitosan Nano-materials has been used as an antimicrobial coating. either antioxidant properties and the ability to interfere with all pathogens [12]. Since it has good tissue-adhesive

Combination of biological agents and Nano-delivery material like Nano chitosan causing Higher drug efficiency by good penetration and distribution , sustained drug relief, improved drug

stability, and improved bioavailability are advantages to prevent biofilm formation in chronic wounds and otherwise reveals anti-bacterial and anti-inflammatory possessions [13].

the potential and properties of nanomaterials and microbial compounds in refining the process of wound and scar healing. So its designate more effective or multivalent wound healing natural or nano-based drugs and wound dressing [14].

Materials and Methods

All the steps of this study Were approved under ethics of Committee of Veterinary Medicine College under NO.1183 date 17/3/2026. Sixty Newzeland rabbits were concluded in this study which divided randomly in three groups, 15 rabbits for each brought from private marketing in plastic boxes, fed a standard food and adlibitum clean Water.

200mg of medium molecular weight, 85% deacetylate chitosan (Sigma Chemical, St. Louis, USA 9012-76-4) was mixed with (200ml) of deionized water and then subjected to sonication and stirring for thirty minutes at room temperature to produce Nano chitosan. The solution was adjusted to a pH of 10 by adding sodium hydroxide and stirring it for an hour; this process will cause the color to become hazy .After the solution was brought to a pH of 4 with hydrochloric acid and agitated for an hour, its color changed to clear. The pH was then raised to 7 using sodium hydroxide.

Klebsiala Pneumonia broth 10 ml Was Prepared on nutrient agar pend deionized Water l0 ml. Were added introduced in magnetic field inductor for 0ne hour. The mixture Was centrifuged 3000 rpm for 5 minutes the filtered to exclude the cells debris and purification the DNA. Then exposed to low level laser pulsating, at 68o nm, 8J. for 10 minutes.

The Field Emission Scanning Electron Microscopy (FE-SEM) micrograph (Inspect S50-FEI) captured at a magnification of 120,000\times clearly reveals the formation of quasi-spherical bacterial DNA nanoparticles. The measured particle diameters ranged from 32.67\text{ nm} to 60.12\text{ nm}, confirming the successful synthesis of sub-100 nm nano-DNA structures. The morphology exhibits well-defined granular clusters, indicating a photothermal/electromagnetic-induced self-assembly and condensation process of the isolated Klebsiella pneumoniae genomic DNA following magnetic field and low-level laser (680\text{ nm}) exposure.

DNA Was measured its concentration by Nano drop, DNA Samples Were examined SEM , UV-spectrum, XRD and FTIR analysis. (Fig,1)

The rabbits were divided in to 4 groups; G1 control group , G2 treated with Nano DNA, G3 Nano chitosan and G4 treated by mix of Nano DNA and Nano chitosan

Complete wounds 2 Cm in diameter Were done under combination injected I/M as general anesthesia. Then treated topically and daily for 7 days. Diameters of Wounds Were measured daily. Biopsies Were collected at 7, 14, and 21 days for histopathological evaluation,

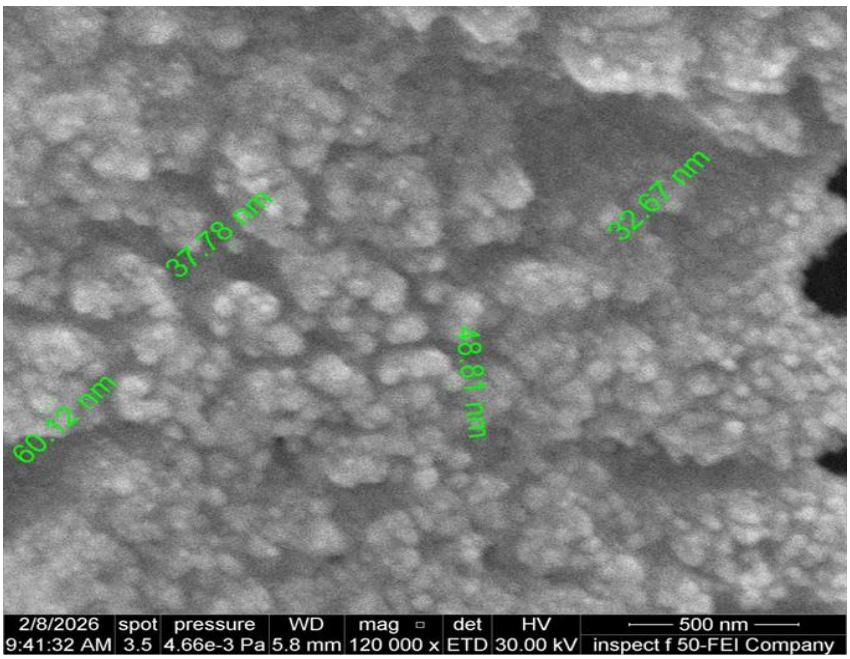

Figure 1. show the elctomicroscopic picture of Nano DNA

Result

All animal of The experimental survive from death and return to normal condition within several days . The treated groups with combination of DNA –chitosan was the first reached healing. This was followed by the group treated with Nano-chitosan group then DNA group while Control group was the last to achieve healing [15].

Skin wound Diameter

Immediately after injury, all groups had a wound circle diameter of 2cm. Decreasing size of wound is noted in the study within several day .

On Day 7, the mean value for wound diameter was measured to be 1.58 ±0.13cm in Control group, 1.48 ±0.08cm in DNA groups, 1.32 ±0.13 cm in Nano-chitosan and 1.24 ±0.09cm in combination groups . After Day 14, the wound diameter decreased in the Control group to 1.28 ±0.08cm, in DNA groups reach to 1.00 ±0.12cm while in Nano-chitosan groups 0.64 ±0.11cm and 0.46 ±0.06cm in combination groups . at the 3rd week of treatment , the decrease in the size of wound in combination groups was (0.08 ± 0.08cm) was the best healing and it was followed by Nano-chitosan (0.32 ±0.08cm) then DNA groups (0.48 ±0.08cm) [16]. While, Control had the biggest lasting lesion (0.68 ±0.16cm). Statistical analysis revealed significant differences ($P < 0.05$) between treatment groups and Control and healing was statistically enhanced in the DNA- chitosan groups compared with other groups .(Table 1.), (fig. 2)

The average Diameter of wound circle in cm were presented in Table (1)

Groups	Day 1	Day 7	Day 14	Day 21
Control (G1)	2 ±0.00	1.58 ±0.13	1.28 ±0.08	0.68 ±0.16
DNA (G2)	2 ± 0.00	1.48 ±0.08	1.00 ±0.12	0.48 ±0.08
Chitosan (G3)	2 ±0.00	1.32 ±0.13	0.64 ±0.11	0.32 ±0.08
Chitosan +DNA (G4)	2 ±0.00	1.24 ±0.09	0.46 ±0.06	0.08 ± 0.08
LSD 21	0.168			

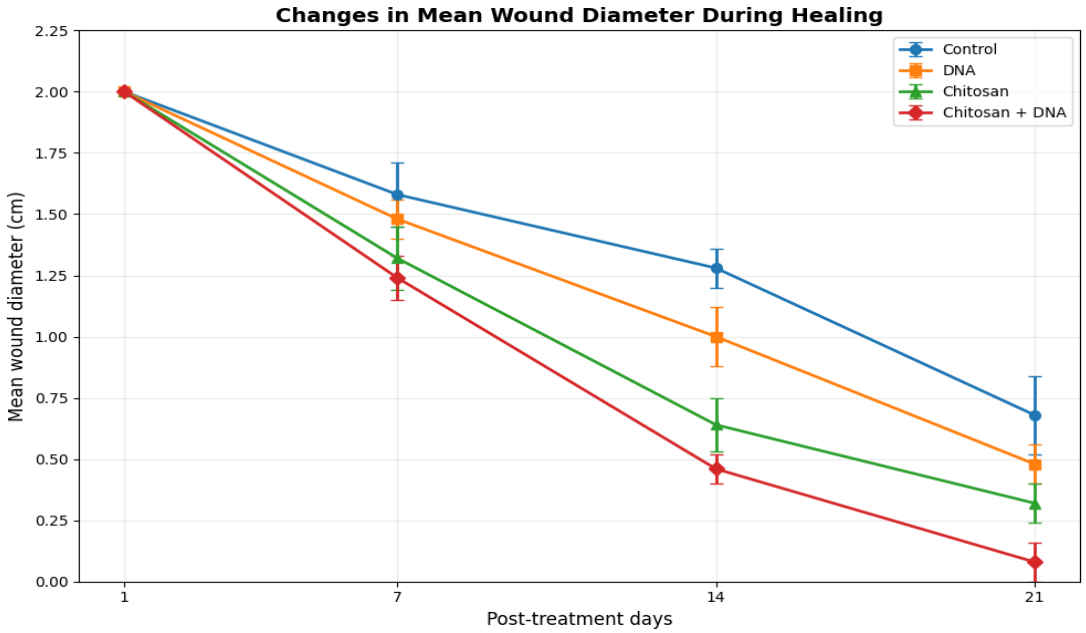

Figure 2. the average Diameter of wound circle.

Microscopic evaluation

Control group (G1):

The histological picture of wound in G1 at 1st week showed stratified squamous keratinized layer site at the epidermis. high infiltration by inflammatory cells with blood vessels and hair follicles these advance indicating that the inflammatory phase of wound healing was current with limited change (Fig.3). at the 2nd week seen complete epithelialization , decreased number of inflammatory cells either seen granulation tissue with irregular of collagen fibers and characteristic by proliferation of endothelial cell, new blood vessels as in (fig. 4) [17]. While, Histological View at 3rd week duration of control group shows formation new blood vessels and clear Granulation tissue and scattered inflammatory cells infiltration with hair follicles seen as in (fig.5) [18].

DNA-treated group (G2):

The histologic evaluation of In G2 demonstrated more radical changes in healing characterized by re-epithelialization, keratinization, profuse granulation tissue, and marked new blood vessel formation Fig,(6). while at 2nd week the histological findings showed heavy in inflammatory cells infiltration and granulation tissue at derma site by fibrous with epithelialization and keratinization s as showed in Fig.(7). And at 3rd week revealed epithelialization keratinization, either increase granulation tissue and inflammatory cells infiltration underlying dermis as showed in fig.(8) [19].

Chitosan group (G3):

The histological findings of wound site of G3 at 1st week showed area of scattered inflammatory cell infiltration, either granulation tissue formation by fibroblast proliferation and increasing of fiberous tissue as in Fig(9) [20]. While at 2nd week showed scattered inflammatory cells infiltration. and replacement of granulation tissue by fibrous with increase fibroblasts epidermis with stratified squamous keratinized epithelium and dermis with hair follicles seen as showed in Fig.(10). Histological sections at wound at 3rd week revealed in complete epithelization , epidermis with the new stratified squamous keratinized epithelium at the dermis seen hair follicles, new blood vessels , heavy fibrous tissue formation and scattered inflammatory cells infiltration as showed in Fig.(11) [21].

Chitosan and DNA (G4):

The section of G4 at 1st week showed area re-epithelialization and keratinization also there was granulation tissue formation, and numerous new blood vessels as showed in fig.(12). While at

2nd week it showed extension of epidermal layer epithelization and keratinization and replacement of granulation tissue by fibrous with scattered fibroblasts and complete epithelization with signs of remodeling accompanied by active fibroblasts and formation new blood vessel and proliferation of epidermal cells as showed in fig.(13). and at 3rd week it showed completed healing of incision which characteristic by complete epitheliazation , keratinization and dermis with less granulation tissue the new epithelium was thin with no rete-ridges, healed wound site with fibrous connective tissue with reduced cellular component could be detected, either showed epithelial cell layers of the new epidermis and few fibroblasts were seen scattered throughout the fibrous connective tissue of the underlying dermis as showed in fig.(14) [22].

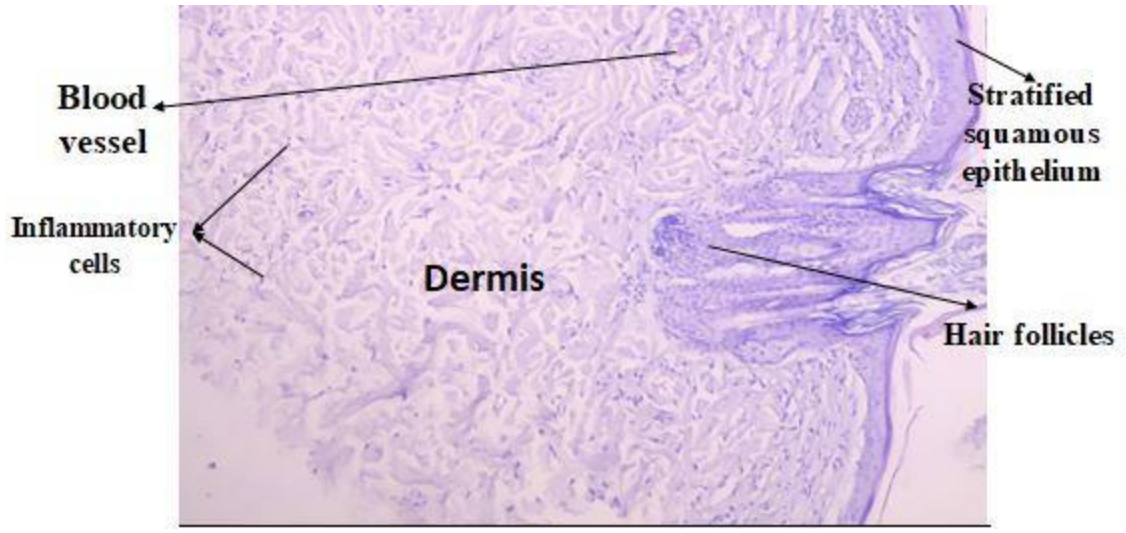

Figure 3. (G1 w1) histological section of skin showing epidermis with stratified squamous keratinized epithelium and dermis with hair follicles seen, and scattered inflammatory cells infiltration with blood vessels. 10X, H&E stain

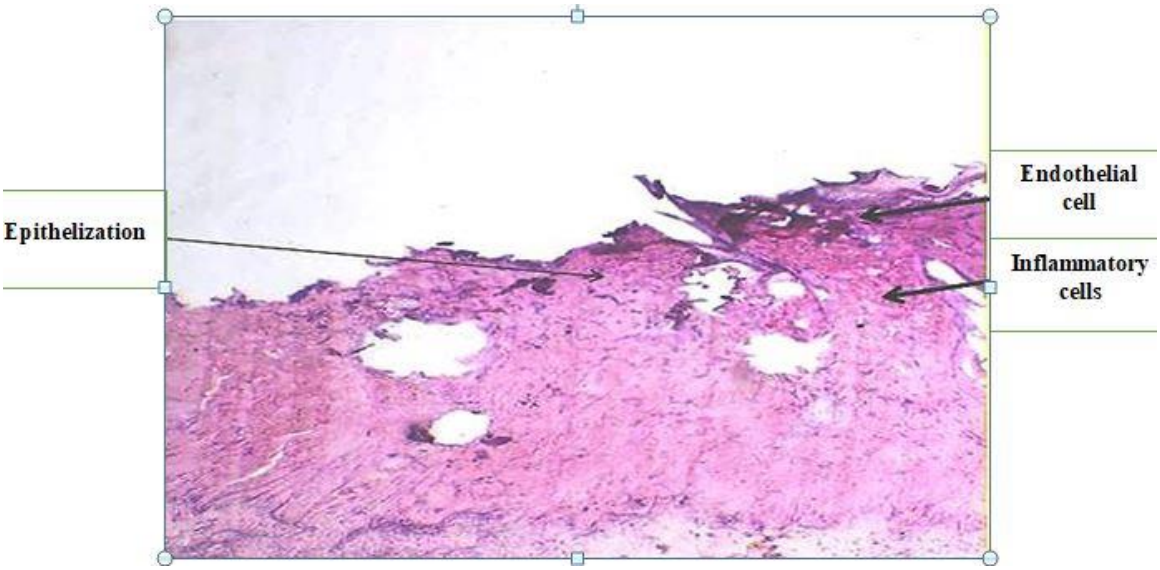

Figure 4. (G1 w2)The histological section from shows a complete epithelialization with a reduced number of inflammatory cells along with granulation tissue characterized by irregular collagen arrangement, endothelial cell proliferation 10X, H&E stain

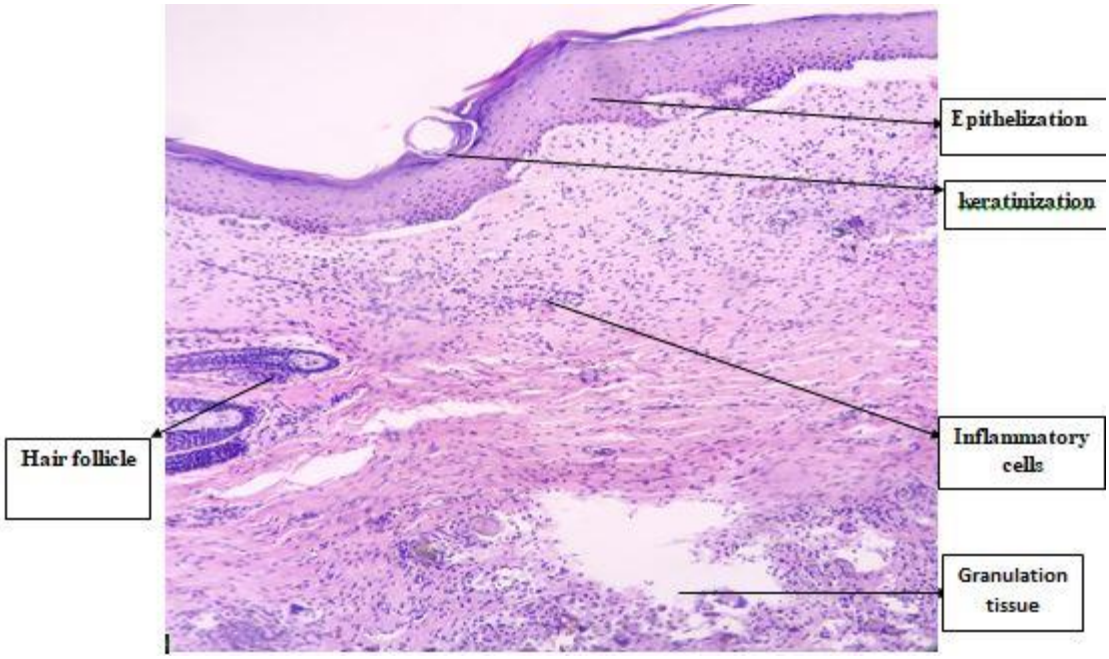

Figure 5. (G1 W3.) The histological section from. of skin showing epidermis with stratified squamous keratinized epithelium and dermis with hair follicles seen, clear Granulation tissue and scattered inflammatory cells infiltration with blood vessels10X, H&E stain

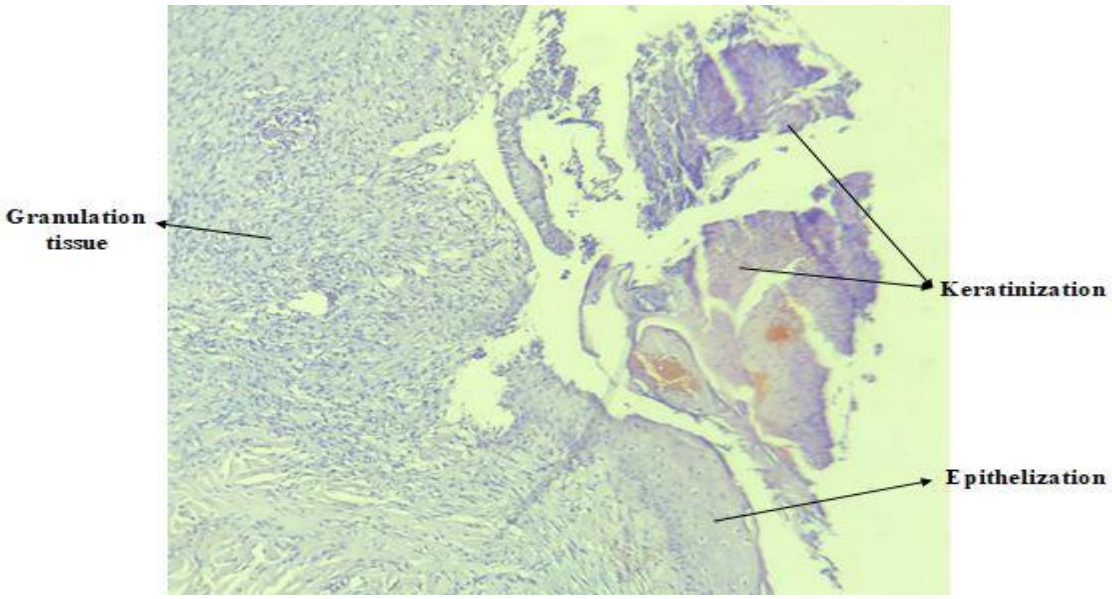

Figure 6. (G2 w1): histological section of skin showing epidermis with epithelization and keratinization and dermis with heavy granulation tissue and new blood vessels formation10X, H&E stain.

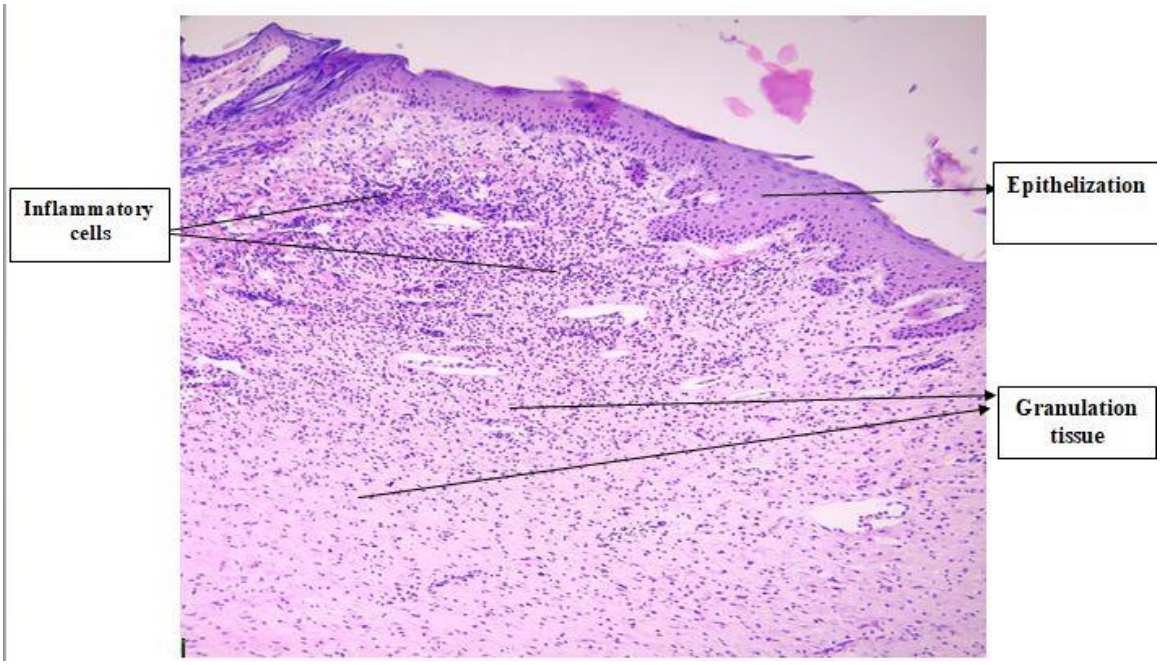

Figure 7. (G2 w2): histological section of skin showing epidermis with epithelization and keratinization and dermis with heavy granulation tissue and heavy inflammatory cells infiltration. 10X, H&E stain

Figure 8. (G2 w3): histological section of skin showing epidermis with epithelization and keratinization and dermis with increase granulation tissue and increase inflammatory cells infiltration10X, H&E stain.

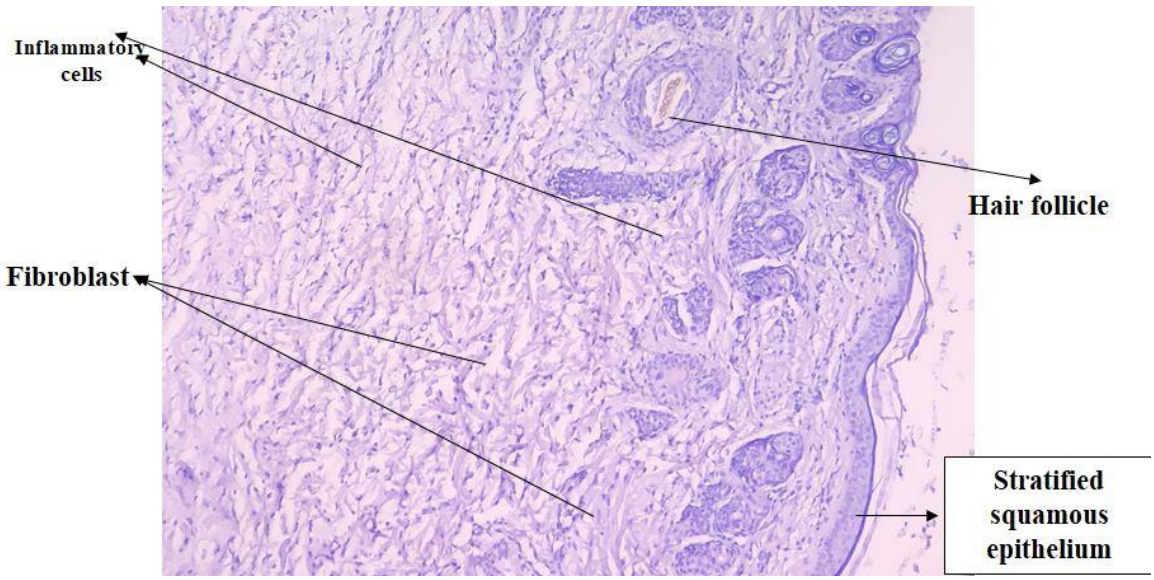

Figure 9. (G3 W1); histological section of skin showing epidermis with stratified squamous keratinized epithelium and dermis with hair follicles seen, increase fibrous tissue formation and scattered inflammatory cells infiltration10X, H&E stain.

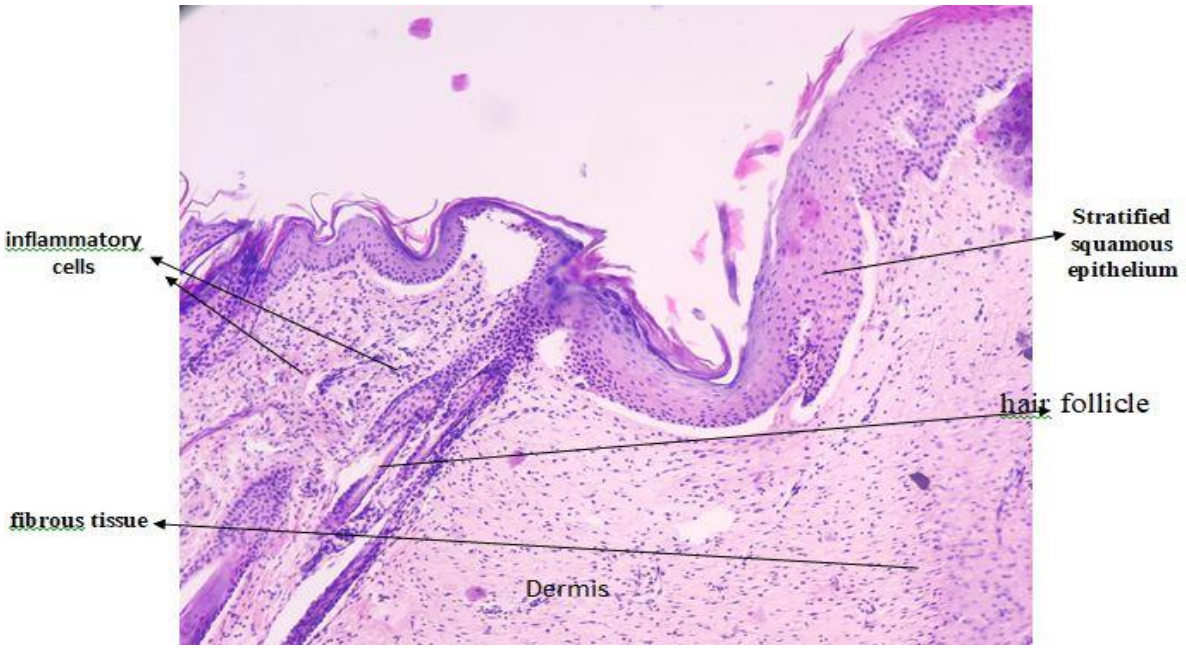

Figure 10. (G3 W2); histological section of skin showing epidermis with stratified squamous keratinized epithelium and dermis with hair follicles seen, increase fibrous tissue formation and scattered inflammatory cells infiltration10X, H&E stain.

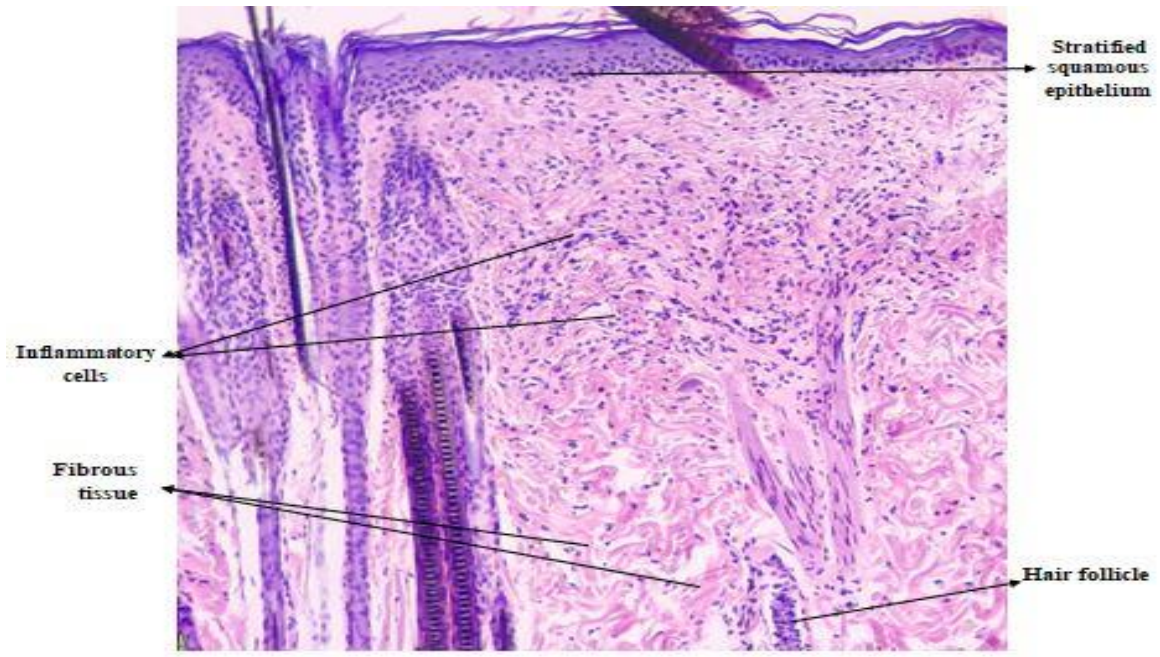

Figure 11. (G3 W3); histological section of skin showing epidermis with stratified squamous keratinized epithelium and dermis with hair follicles seen, heavy fibrous tissue formation and scattered inflammatory cells infiltration10X, H&E stain.

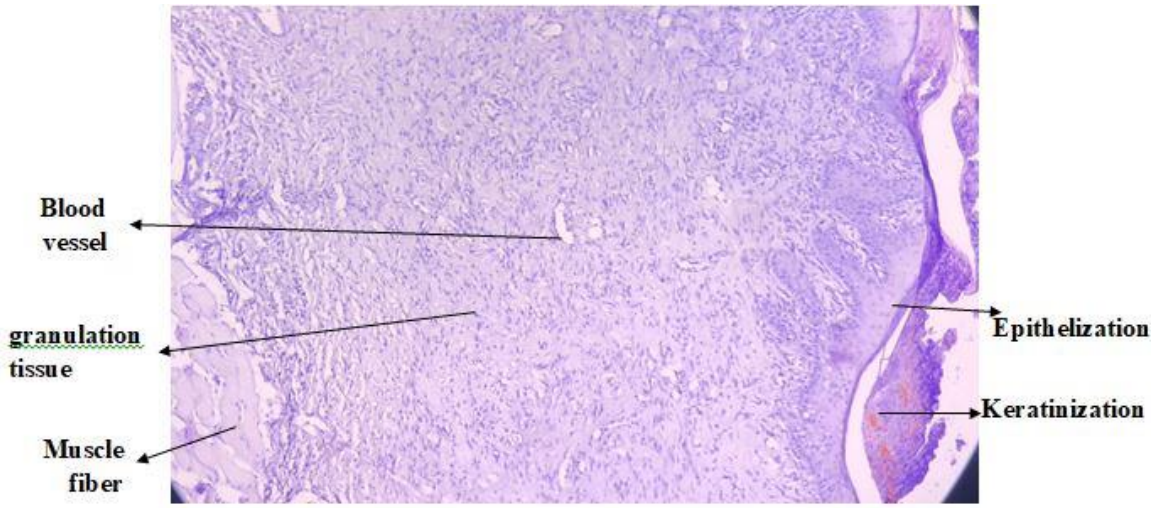

Figure 12. (G4 W1); histological section of skin showing epidermis with epithelization and keratinization and dermis with fewer granulation tissue and less new blood vessels formation.

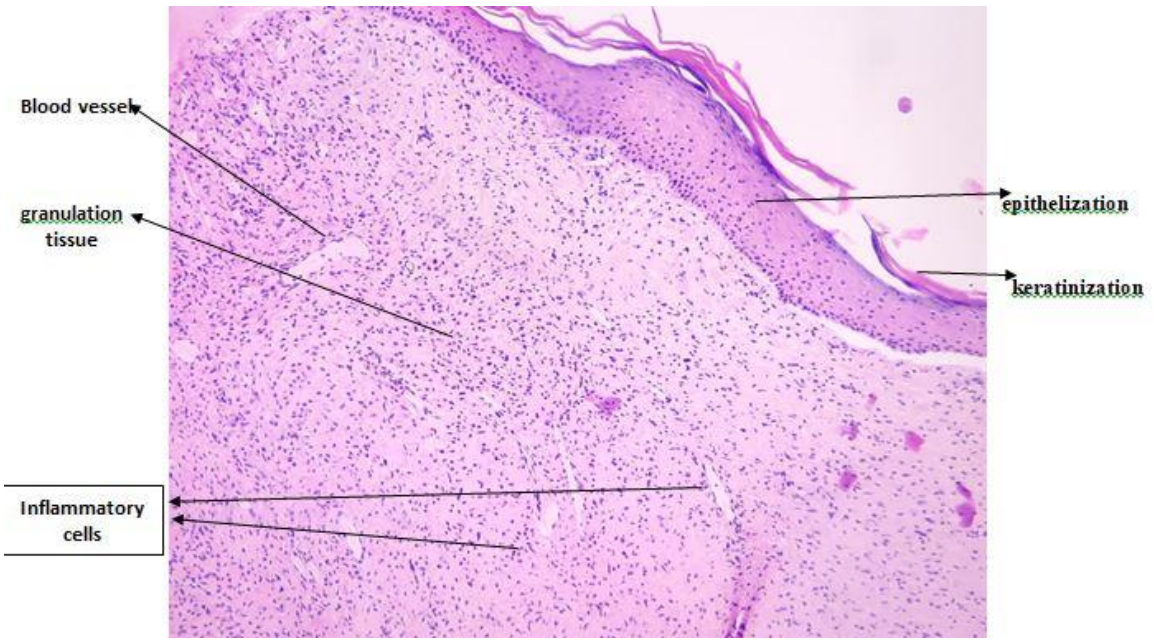

Figure 13. (G4 W2); histological section of skin showing epidermis with epithelization and keratinization and dermis with less granulation tissue and new blood vessels formation with inflammatory cells infiltration. 10X, H&E stain.

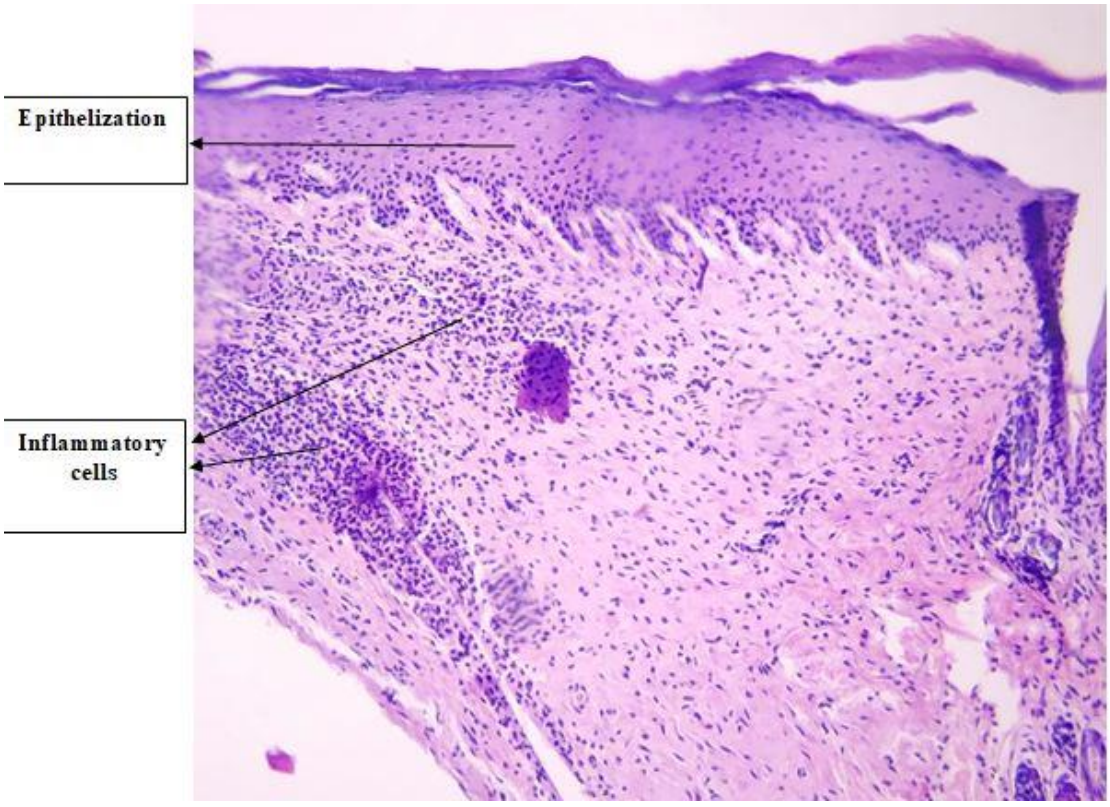

Figure 14. (G4 W3); histological section of skin showing epidermis with epithelization and keratinization and dermis with less granulation tissue and sever inflammatory cells infiltration and few fibroblasts were seen scattered throughout the fibrous connective tissue of the underlying dermis. (10X, H&E).

Discussion

As definite recently, wound healing retires from a combination of chronic infection, biofilm formation and a aggressive inflammatory microenvironment that delays the normal sequence of inflammation, granulation, tissue formation, angiogenesis, and remodeling [23].

Wound or burn healing is a tightly coordinated biological process that proceeds through inflammation, proliferation by granulation tissue formation and angiogenesis, and remodeling [24]. Any factor that interrupts this series, such as decreased local metabolism, infection, or biofilm formation, delays healing and changed to chronic, non-healing wounds. The present histological findings across the four experimental groups support this framework and clarify how Nano chitosan, DNA nanostructures and their combination modify each phase of repair [25].

Control group G1

In this group, healing followed the expected,; a prolonged inflammatory phase at week 1 with dense WBCs infiltration and few epithelial development, gradual replacement of granulation tissue by structured collagen by week 2, and re-epithelialization with vascular remodeling only by week 3. This slow, stepwise development characterizes the natural phase of tissue repair in the lack of any bioactive intervention and serves as the starting point contrary to which the treated groups can be refereed [26].

DNA treated group (G2):

Observation in the DNA- group (G2) the wound smaller than control, (0.68_+ 0.16 cm) but still larger than the chitosan and combination groups. This is reflected , DNA nanostructure were effect on major obstacles to wound closure. Microscopically, showed an accelerated onset of re-epithelialization, keratinization and marked angiogenesis as early as week 1, that confirm by whom say that framework DNA (FNA) nanostructures enhance re-epithelization by moderating the cross linkage between macrophages, fibroblasts and keratinocytes and that DNA are effective dressings for difficult, chronic-type wounds [27]. Particularly, however, inflammatory cell infiltration remained heavy through 2nd weeks and 3rd rather than solving in parallel with the epithelial changes. This dissociation suggests that DNA nanostructures may act primarily on the regenerative , angiogenesis axis of healing rather than by directly dampening the inflammatory response, and it may explain why complete, mature epithelization in G2 lagged behind the combination group despite an early proliferative head start [28].

Nano-chitosan (G3):

Nano-chitosan showed a more effect than DNA alone (table 1), consistent with its documented multifunctional properties large surface area, tissue adhesiveness, biodegradability and Its strong antibacterial activity, along with its antioxidant properties, likely contributed to reducing the infective and inflammatory burden that otherwise delays healing in wounds[29]. Either chitosan, accelerated transition from inflammation to proliferation; scattered rather than dense inflammatory infiltrate from week 1, active fibroblast proliferation with extracellular matrix (collagen) deposition, and replacement of granulation tissue by fibrous tissue with hair follicle regeneration by week 3. These findings agree with the established biological properties of chitosan, its biodegradability, hemostatic and tissue-adhesive nature, and its ability to promote fibroblast proliferation, granulation tissue formation, and neovascularization while keeping the wound bed moist and protected. Its additional antimicrobial activity, particularly against gram-negative organisms, together with its antioxidant and anti-biofilm properties, likely contributed to controlling local infection and thereby indirectly supporting the more moderate, better-regulated inflammatory response observed histologically [30].

Combination group (G4):

The best result in the combination group (G4) supports the concept, raised in the introduction, that pairing biological\Nano-delivery agents can yield higher drug effectiveness through improved penetration. Combining chitosan's antimicrobial, anti-inflammatory and scaffold-

forming properties with DNA nanostructures regenerative and anti-biofilm signaling appears to address the three challenges of chronic wounds, infection, inflammation and impaired regeneration, simultaneously rather than individually [31]. The combined chitosan-DNA group G4 showed the most favorable healing profile of all groups; early re-epithelialization and keratinization with granulation tissue and neovascularization at week 1, fibrous remodeling with active fibroblasts and ongoing angiogenesis at week 2, and by week 3 essentially complete healing, a tinny, epithelium layer with minimal residual granulation tissue and a well-organized, less cellular fibrous dermis. This superior consequence is reliable with the idea that combining a Nano-delivery scaffold such as chitosan with a biologically active nanostructure such as DNA yields a synergistic effect; chitosan provides a protective, antimicrobial, pro-adhesive structural matrix with sustained and improved bioavailability of any combined agent, while DNA nanostructure supply the regenerative and antigenic signaling needed to drive re-epithelialization. Together the appear to support the broader concept raised in the introduction that multivalent, nanomaterial-based wound dressings, rather than single-agent therapies, represent the more effective strategy for both acute and chronic wound management. Overall, these findings suggesting that nanomaterial-based dressings, especially those combining multiple functional nanostructures, signify accelerating wound closure.

Conclusion

The combination of chitosan and DNA nanostructures achieved the fastest and complete closure among the tested groups , followed by Nano-chitosan, DNA nanostructures, while the untreated control group showed the slowest healing and the largest residual lesion at day 21. These findings support the potential of chitosan-DNA nano composite dressings as benefit for accelerating wound healing,

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