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ROLE OF NANO FIBER SCAFFOLDS IN WOUND HEALING

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ABSTRACT
Background: Chronic wound remains a global healthcare problem for patients who suffer from burns or chronic skin ulcers. Wound healing is a natural restorative response and its purpose is to repair the skin to prevent infection and to restore tissue integrity and function. Management of chronic wounds includes the application of an antimicrobial dressing which reduces inflammation and regulation of other pathogens. Fiber based dressing provides moist environment, removes excess exudates, allows gaseous exchange, and does not release fiber materials to the wound hence minimizing the risk of infection. The objective of the present study is to evaluate the role of chitosan based nano fiber scaffolds in wound healing. **Methods:** A prospective, randomized, interventional trial that was conducted at the Department of Plastic Surgery Thanjavur Medical College, Thanjavur, India between January 2018 and December 2018 to evaluate the potential of chitosan based nano fibers in promoting skin regeneration in various skin wounds. A total of 56 patients who fulfilled the inclusion and exclusion criteria were recruited for the trial. The treatment group of 30 patients received the chitosan based nano fiber dressing and the control group of 26 patients received traditional dressing in the form of gauze, bandage and adhesive tapes for 20 days. **Results:** In the control group four wounds healed but the remaining wounds all deteriorated and became infected, requiring antibiotics. In contrast, in the treatment group 26/30 wounds healed completely, and none became infected; the remaining four wounds healed during the follow-up period. The difference between the two groups in the number of wounds that healed was statistically significant ($p<0.001$). **Conclusions:** Chitosan, an important biodegradable and biocompatible polymer possess antimicrobial properties such as antibacterial, antifungal and other physical and chemical properties which enhance the wound healing in a better and faster closure.

KEYWORDS: Nano fiber, Wounds, Skin regeneration.

INTRODUCTION
Chronic wounds are a major healthcare issue. Current estimates indicate that nearly 6 million people suffer from chronic wounds worldwide. In India the prevalence of chronic wounds in the com-munity was reported as 4.5 per 1000 population where-as that of acute wounds was nearly doubled at 10.5 per1000 population.[1] Chronic wounds afflict a very large number of patients and seriously reduce their quality of life. In the United States alone, 1.25 million patients suffer from burns and an additional 6.5 million patients endure chronic skin ulcers most commonly caused by pressure, venous stasis, or diabetes mellitus.[2]

A wound dressing is a protective barrier used to assist in many aspects of the healing process. In comparison to typical bandages, which do not meet all the requirements of wound care, electro spun fiber mats could potentially provide an excellent environment for healing. The ideal goal of skin tissue engineering is to restore the structural and functional properties of wound site.

Recent research on skin tissue engineering is focused on biomimetic nano fibrous scaffolds to treat various skin wounds.[3] Nano-geometry can support cell adhesion and proliferation. Electro spinning is one of the widely used techniques to fabricate nano fibrous scaffolds for tissue engineering. The clinical efficacy of nano fibers in treating various skin wounds has not yet been explored. Therefore, this work presents the investigation of the potential of nano fibrous scaffold in treating various wounds like traumatic wounds, surgical wounds, burns, diabetic wounds, wound bed with poor vasculature and non-healing ulcers.

METHODS

A prospective, randomized, interventional trial was conducted at the Department of Plastic Surgery, Thanjavur Medical College, Thanjavur, India between January 2018 and December 2018 to evaluate the potential of chitosan based nano fibers in promoting skin regeneration in various skin wounds. A total of 56 patients who fulfilled the inclusion and exclusion criteria were recruited for the trial.

Inclusion Criteria: Males and females between 18 and 65 years of age, body weight within the appropriate range, patients on no regular medical treatment for wound healing / diabetic wound healing and able to communicate effectively with study personnel were taken up for the study.

Exclusion Criteria: Any disease or condition which might compromise the haematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system, history of allergic conditions – asthma, urticaria, eczema, history of autoimmune disorders, history of psychiatric disorders and recent history of alcoholism (<2 years) and smokers were excluded from the study.

Ethics committee approval was obtained and all patients gave written informed consent. 30 patients were randomised to receive the chitosan based nano fiber dressing (treatment group) and 26 patients received traditional dressing in the form of gauze, bandage and adhesive tapes (control group).

Preparation of Nano fiber Scaffold: Chitosan, Poly vinyl alcohol (PVA) were used as polymer and acetic acid was used as a solvent. Poly (3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) nano fibers were fabricated by using electro spinning technique (Figure 1). Co-solvent system containing dichloromethane (DCM) and dimethylformamide (DMF) (9:1 ratio) was used to prepare PHBV solution. A glass syringe (5 mL) with 26 gauge blunt needle was used to load the PHBV solution. The polymeric solution was pumped at a flow rate of 0.002 mL/min with the help of a syringe pump. The PHBV solution was subjected to high voltage (19 kV) and the nano fibers were deposited onto a grounded static collector placed 12cm away from the syringe tip. The electro spun nano fibers were vacuum dried for 72 hours. Then the grafts (0.2 mm to 0.3mm thick) were individually wrapped and subjected to ethylene oxide sterilization (Figure 2).

Study Procedure: Baseline data included the patient's gender, age, weight, comorbidities, the wound aetiology, duration, location, size, stage and presence of infection. Wound was cleaned with distilled water. Mechanical debridement was performed to eliminate loose slough and necrotic tissue during the cleaning. Pus culture was done. Under strict aseptic precautions all the wounds in both treatment and control groups were inspected. Wounds in the treatment group were irrigated with normal saline and photograph of the type and grade of the wound was documented. The wound was then covered with PHBV nano fibrous mat and a non–adherent pad and fixed with a polyurethane adhesive. Patients with heavily exuding wounds had their dressings changed every other day, and those with medium orl ow exudate had dressing changes every four days. Patients in the control group received standard wound care in accordance with the hospitals' protocol. Wounds were irrigated with normal saline and covered with gauze secured with a bandage and adhesive tape. Dressings were changed once or twice daily, depending on the exudate level. Wound Assessment in both groups: Wound size and grade were assessed with photographs. The exact length and width were calculated using J software. Wounds were swabbed if they showed clinical signs of infection. The treatment period for both groups was 20 days, during which patients could be discharged from hospital. Patients were then regularly followed up and the wound size and grade were assessed. The rate of wound healing and the presence of infection were the primary outcomes of the study. Statistical analyses were done using analysis of variance (ANOVA) and chi-square test, using SPSS software. A p value of <0.05 was considered significant.

RESULTS

Among the 56 patients recruited for the trial, 34 had traumatic wounds, 12 post burn injuries and 10 diabetic wounds. The sample comprised 20 females and 36 males, with a comparable male: female ratio in the two groups. The mean age in the control and treatment groups was 44.8 and 48.6 years respectively.

Treatment Group: Of the 30 patients in the treatment group 20 patients had post traumatic wounds, 6 patients had post burn wounds and 4patients had diabetic wounds. None of the patients treated with nano fiber dressing reported to have any adverse reactions (itching, burning, and swelling/inflammation) in the wound bed. Patients experienced comfort to the wound bed after the application of nano fiber dressing. The healing duration ranged from 14 to 55 days depending on the size and depth of the wounds (Figure 3). Traumatic wounds in the treatment group exhibited more than 90% healing within 40 days of treatment. The acceleration of wound healing with nano dressing in most of the wounds started after three weeks. In one patient the healing started rapidly after 40 days and completed within 48 days (Figure 4). Histopathology results confirmed normal re-epithelialization with abundant angiogenesis. The rate of healing in the post burn and diabetic wounds of the treatment group were also high when compared to the control group (Figure 5). Interestingly, there was minimal scar formation in most of the treated wounds (Figure 6). Percentage healing in this group was significantly higher when compared to the control group ($p<0.05$) (Figure 7). The nano dressing neither dissolved during the application period nor adhered to the wound and was easy to remove without ripping the skin.

Control Group: Of the 26 patients in the control group 14 patients had post traumatic wounds, 6 patients had post burn wounds and 6 patients had diabetic wounds. In the control group four wounds healed but the remaining wounds became infected and required antibiotics. During the study 20 wounds became infected (>105 CFU/ ml), with polymicrobial growth; an additional two wounds revealed beta-haemolytic streptococcus (>103 CFU/ml). All of these patients received antibiotics.

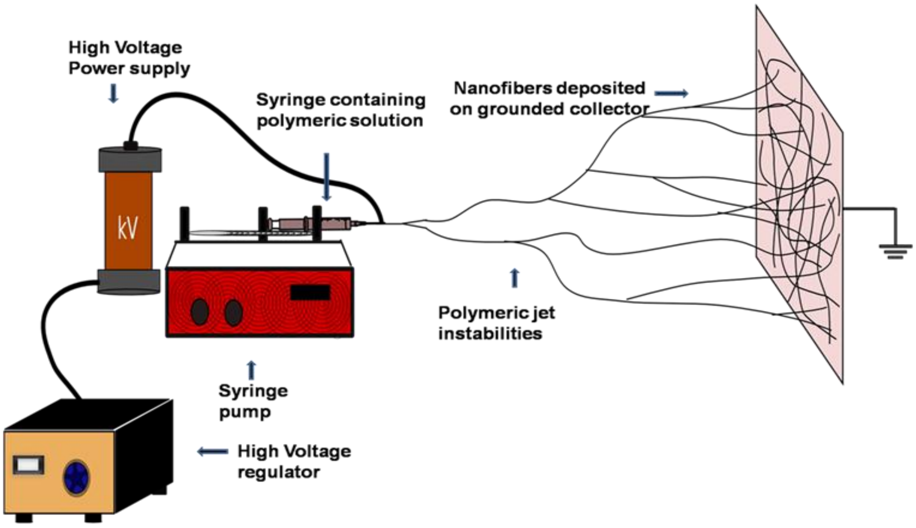

Figure 1: Electro spinning.

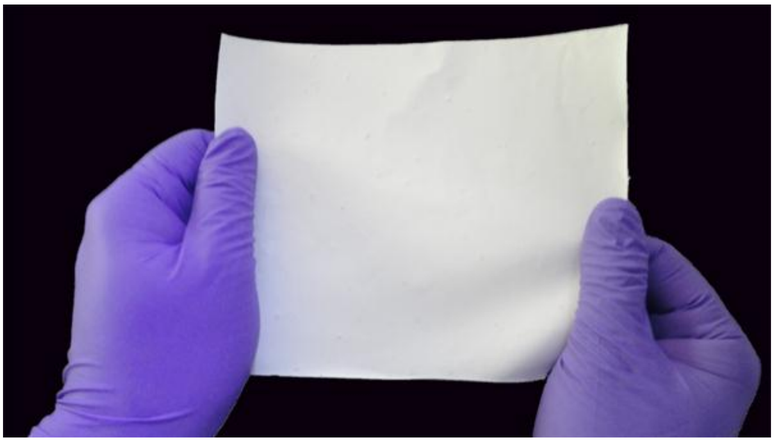

Figure 2: Electro spun PHBV Nano fibrous Mat.

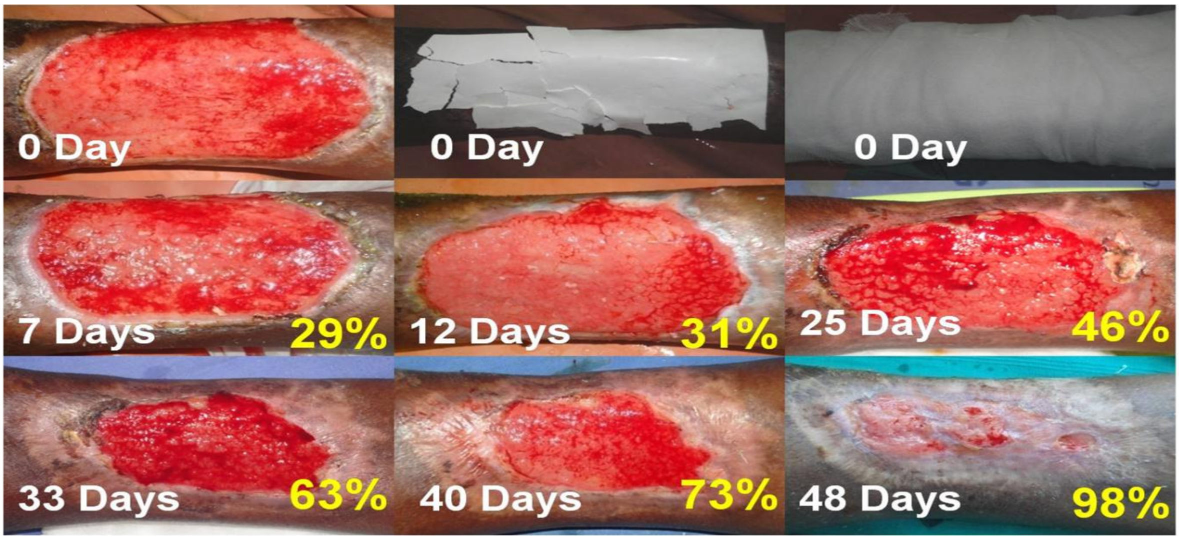

Figure 3: Traumatic wound of female with nano dressing.

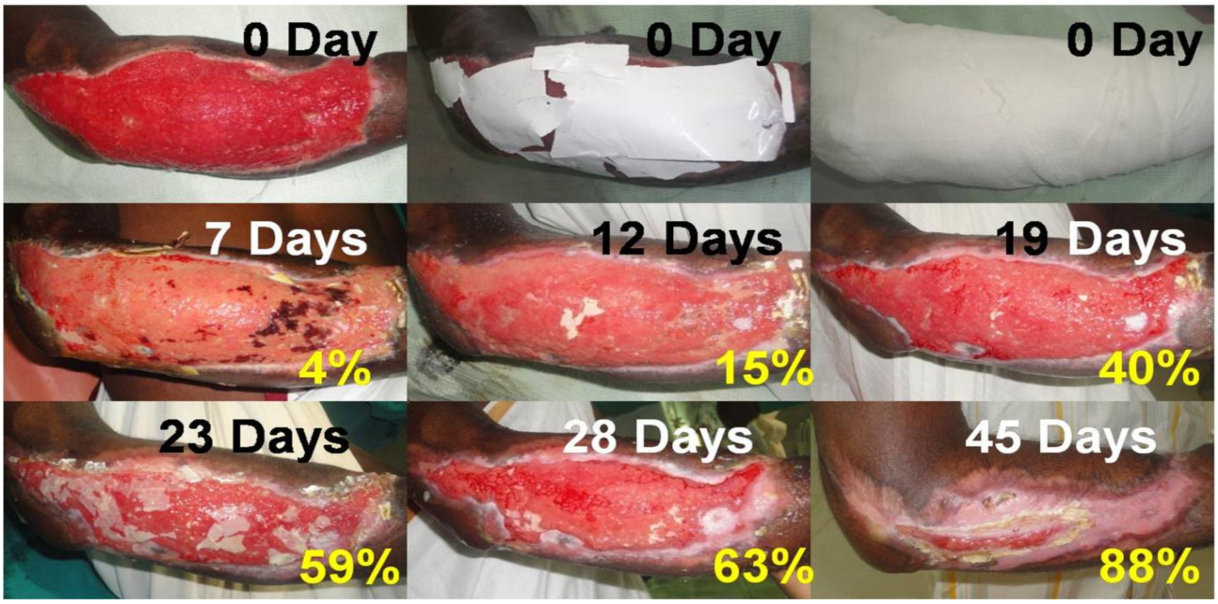

Figure 4: Traumatic wound of forearm with nano fiber dressing.

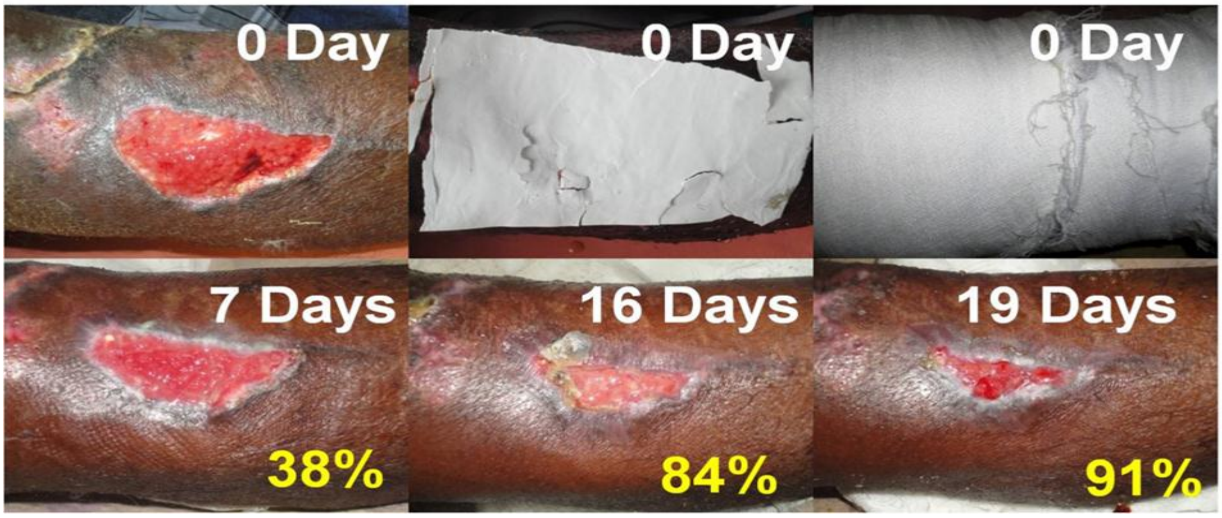

Figure 5: Diabetic wound healing with nano dressing.

Figure 6: Minimal Scar in the treated wound.

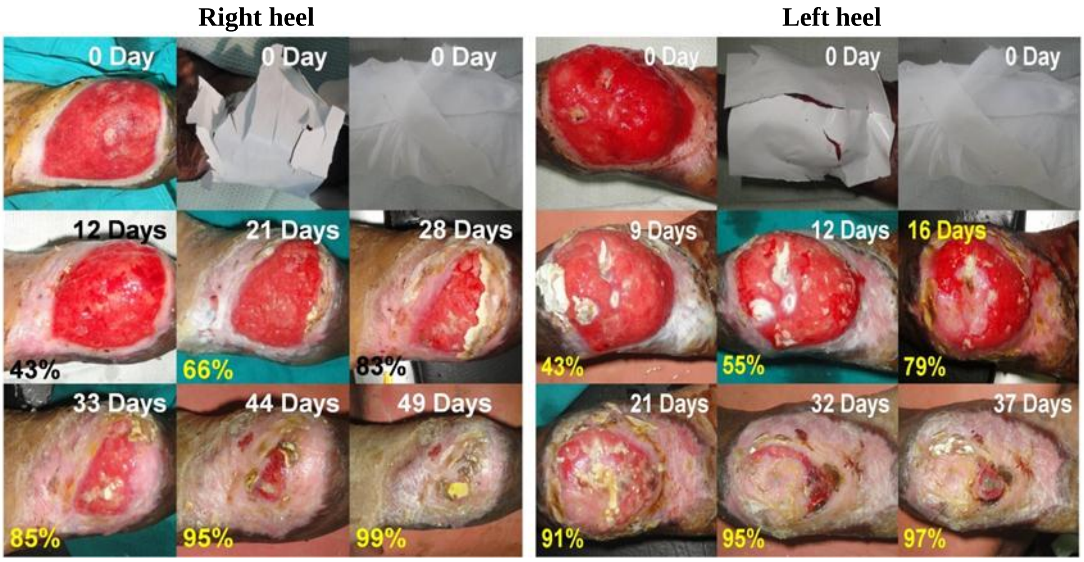

Figure 7: Traumatic wound on both heels with nano fiber dressing.

DISCUSSION

Chronic wounds afflict a very large number of patients and seriously reduce their quality of life. The most difficult aspects of chronic wounds are the prolonged healing times, risk of infection, deterioration of the patient's general health and weakening of the immune system, which can increase morbidity and even result in death. Chronic wounds like diabetic ulcers fail to heal and finally end up with amputation. Similarly in extensive burn wounds the donor area availability is restricted for repeated grafting. Hence an alternative method for the healing of chronic wounds is needed.

Various materials such as hydrocolloids, alginates, and hydrogels derived from different base material sources have been explored as potential biomaterials for advanced wound therapy. The most commonly used natural polymers in the making of natural biomaterials for wound management are proteins and/or polysaccharides, or derivatives of the two.[4]

An alternate to collagen-based natural biomaterials for wound healing is chitosan. Chitosan is produced by de acetylation of chitin, which is the structural element in the exoskeleton of crustaceans such as crabs, lobsters and shrimps.[5] The shells of these are removed and ground into powder which is then de acetylated, producing chitosan, a compound widely used in the cosmetic and food industries and in medicine. Many studies have demonstrated the therapeutic properties of chitosan, such as alleviating pain, inhibiting growth of microorganisms and promoting haemostasis and epidermal cell growth.[6] Chitosan has great wound healing properties due to its haemostatic effect. Furthermore, it is believed that it increases early-phase, healing-related reactions and promotes fibroblast proliferation.[5,6] The low toxicity of chitosan and its biodegradation products and its high biocompatibility with tissues and blood allow chitosan-based biomaterials to be strong applicants for wound healing and wound management.

Chitosan also possesses a wide range of antimicrobial properties. Three antibacterial mechanisms have been proposed in a research whereby ionic interaction resulted in cell wall leakage, binding chitosan with the microbial DNA leading to the inhibition of the mRNA and protein synthesis, and finally achieving formation of an external barrier, chelating metals and inducing the suppression of microbial nutrients.[7, 8,9,10]

Chitosan will slowly depolymerise and release N-acetyl-β-D-glucosamine which initiates fibroblast proliferation and greatly assists in collagen deposition, thus resulting in faster wound healing and scar prevention.[11,12,13,14,15]

In this study we found that PHBV nano fibers application provided comfort to the wound bed and there was no adverse reaction. Significantly the healing rate was higher when compared to tradionally treated wounds and untreated wounds. Study limitations include the difference in size between the two groups, the short study period, inadequate blinding and the large drop-out rate in the follow-up period.

CONCLUSION

Chitosan, an important biodegradable and biocompatible polymer possess antimicrobial properties such as antibacterial, antifungal and other physical and chemical properties which enhance the rate of wound healing. There is a reduced need for antibiotics. From the outcome of this study it is concluded that PHBV nano fibers can be used as an alternative for chronic non healing wounds.

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DECLARATIONS

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