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Antimicrobial and Wound Healing Properties of Polyacrylonitrile-Moringa Extract Nanofibers

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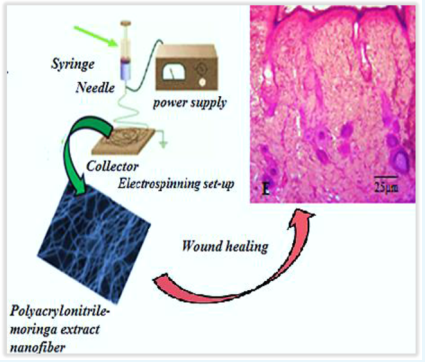
ABSTRACT: A simple and cost-effective material composed of polyacrylonitrile nanofibers containing different concentrations of moringa (MR) leaf extracts was fabricated for antimicrobial properties and wound dressing. The fabricated materials were characterized by scanning electron microscopy, thermal gravimetric analysis, and Fourier transmission infrared spectroscopy. The antibacterial sensitivity of the developed polyacrylonitrile-moringa extract nanofibers was evaluated against *Staphylococcus aureus* and *Escherichia coli* by the agar diffusion method. A pronounced antibacterial activity was observed with the increase in the incorporated moringa leaf extract concentration within the polyacrylonitrile-moringa extract nanofibers against the bacterial strains. The best antibacterial sensitivity was observed for nanofibers containing 0.5 g of moringa leaf extract which had an inhibitory zone of 15 mm for *E. coli* and 12 mm for *S. aureus*. Furthermore, the cost-effective and biodegradable nanofibrous polyacrylonitrile−moringa extract nanofiber was also used to conduct further studies regarding wound dressing. The result reveals that the increase in the concentrations of moringa leaf extract influenced the healing properties of the material. For days 1, 4, and 7 of the wound dressing experiment, the % wound closure of the rat was the highest for the nanofiber containing 0.5 g of moringa leaf extract (35, 87, and 95%, respectively) compared to the positive control medical gauze (29, 75, and 93%, respectively).



1. INTRODUCTION

Polymer nanofibers have been reported to have wide applications in the field of nanotechnology.[1,2] Nanofibers can be produced by using a simple, versatile, and widely applied method known as electrospinning.[3] The morphology of the nanofibers produced can be influenced by parameters such as applied voltage, viscosity of solution flow rate, and distance of the collector from the syringe.[4−7] Nanofibers obtained via this method from different polymers have proven to have potential applications in medicine[8] and environmental applications[9] such as electrochemical sensors for organochlorine pesticides,[10] drug delivery, and food processing.[11] Green synthesis and fabrication of nanofibers with extracts from plants have also been explored.[12−14] Recently, electrospun nanofibers have been used as materials for wound dressing because of high oxygen porosity of the materials and also the possession of different pore sizes, high surface/volume ratio, and similar texture to the natural extracellular matrix in the skin, which promotes the wound healing process.[15−17] Polymer nanofibers can be used either with or without any additives or in combination with bioactive plant extracts.[18,19]

Some of these polymers have properties such as biocompatibility and biodegradability. Biodegradable polymers could be classified into synthetic and natural polymers. Both synthetic and natural biodegradable polymers have been used for drug delivery, and some have been developed for clinical applications. The ease of transformation of some of these polymers into nanofibers has encouraged researchers to explore the possible applications available for these nanomaterials.[20] Polyacrylonitrile falls among the list of very important polymeric materials because of its easy fiber formation by electrospinning with unique mechanical, thermal stability, and good solvent resistance properties.[21] Biodegradability property of some polymers can be improved by reacting them with other

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biodegradable materials.[22] The synthesis and characterization of biodegradable composites of polyacrylonitrile have been reported for various applications and can form composites with other materials including the extract from various parts of plants.[22−25] Polyacrylonitrile has also been used as good anode materials for LIBs,[26] electrodes for coin cell supercapacitors,[27] as biocatalytic O_2 reduction,[28] degradation of rhodamine,[29] and wound dressing.[30]

Plants with antiproliferative, antioxidant, and antimicrobial properties have been used in the treatment of wounds. Examples of such plants are *Aloe vera*, *Moringa oleifera*, and *Kigelia africana*. They are often delivered in the form of ointment on the affected region.[31] Bioactive plant extracts from plants such as *M. oleifera*, a species of monogeneric family Moringaceae,[32,33] contains phytochemicals such as phenolics, zeatin, kaempferom, quercetin, and amino acids. Specifically, the protein isolates from the *M. oleifera* leaf, which is confirmed to be a biodegradable film consists of niazirin, niazirinin, 4-[4′-*O*-acetyl-α-L-rhamnosyloxy) benzyl-isothiocyanate, and niaziminin A and B.[34,35] Traditionally, the plant has found applications in pharmaceuticals such as cardiac and circulatory drugs and antipyretic, antiulcer, antiinflammatory, antiepileptic, antispasmodic, diuretic, antihypertensive, cholesterol lowering, hepatoprotective, antioxidant, antidiabetic, antibacterial, and antifungal drugs.[36,37]

A wound is described as an injury either to the skin, underlying tissues or organs which are caused by physical, chemical, thermal, microbial, and immunological abuse.[38,39] Furthermore, the wound is usually accompanied by vasoconstriction which induces homeostasis and release of inflammation mediators.[40,41] Another major concerns with wound is the high risk of infections. Bacterial organisms such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Streptococcus* spp are a group of bacteria that often colonizes the wound and cause infections within 48 h[40,41] when exposed. Wound healing is therefore a biochemical and physiological process of repairing the damaged tissue to its almost normal state.[42] Wound healing is usually characterized by reformulations and improvements in the components of the collagen fibers that increase the tensile strength of the tissues.[43−46]

Nanofiber membranes of different polymers such as polyvinylacetate, poly(ε-caprolactone), and polyacrylonitrile have been used as wound-dressing materials. The results showed that the nanofiber membranes were high in mechanical strength and therefore facilitated the healing process.[47] An ideal polymer to be used for wound dressing therefore must possess properties such as noncytotoxic, biodegradable, impermeable to bacteria, hemocompatible, easy to remove, and capable of maintaining the moisture content over the wound surface.[48] Other properties are antibacterial activity, odor-absorbing properties, ability to remove chronic wound fluid, and effective wound-cleansing activity.[48] Polyacrylonitrile with other polymers such as polyvinylchloride was reported to exhibit strong antimicrobial activity against *S. aureus* and *E. coli* that can cause wound infections.[46]

However, the available skin substitutes used in wound healing causes problems such as wound contraction, scar formation, and poor integration with the host tissue.[49] The properties of nanofibers make them suitable for burns and wound healing. Their large surface area also increases the close interaction of therapeutic agents and exchange of oxygen and carbon dioxide with tissues and provides the mechanism for sustained release and delivery of plant-derived remedies, analgesics, antibiotics, and growth factors needed for burns and wound healing.[50]

Therefore, this study reports the fabrication and characterization of polyacrylonitrile nanofibers containing different concentrations of moringa leaf extracts for wound healing. The materials were characterized by Fourier transmission infrared spectroscopy (FTIR), scanning electron microscope (SEM), XRD, and thermal gravimetric analysis (TGA). The wound healing properties of different concentrations of moringa leaf extracts in the nanofibers were investigated using animal models. The antibacterial potentials of moringa leaf extract-functionalized nanofibers against *E. coli* and *S. aureus* bacteria strains were investigated using Agar diffusion methods.

2. RESULTS AND DISCUSSIONS

2.1. Spectroscopy and Morphology Characterization Nanofibers. The results of FTIR spectroscopic characterization of the functional groups present in the electrospun nanofiber of polyacrylonitrile only and nanofibers with 0.5 g concentration of moringa leaf extract are shown in Figure 1.

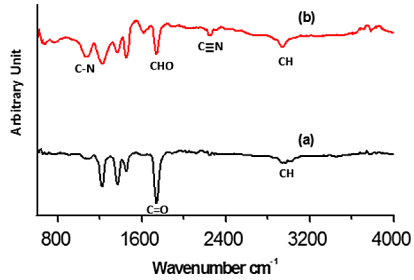

Figure 1. FTIR spectra of 16 wt % (a) polyacrylonitrile only and (b) polyacrylonitrile +0.5 g MR leaf extracts.

The spectra for polyacrylonitrile nanofibers only show absorption peaks at 2949−3014 cm^{-1} attributed to C−H bonds in CH, CH_2, and CH_3,[54] while another peak at 2245 cm^{-1} is the characteristic peak confirming the presence of nitrile C≡N bonds and nitrile groups present in polyacrylonitrile. Peaks observed at 1736 and 1198 cm^{-1} are assigned to C═O or CO, respectively.[55] Other peaks observed are CH stretching at 2933 and 1633−1546 cm^{-1} due to the amide I and amide II regions. Also observed is the amine (C−N) stretch vibrational band around 1050 cm^{-1} and carbonyl group CHO stretching at 1756 cm^{-1}, and the band at 1076 cm^{-1} is the C−O stretching vibration confirming the presence of alcohols and carboxylic acid groups in the moringa leaf extract present in the polyacrylonitrile nanofiber. Similar peaks were observed in the spectra for the other concentrations (graph not shown).

The SEM morphology of the polyacrylonitrile nanofiber and that of polyacrylonitrile with different concentrations of moringa leaf extracts were carried out to observe any change in morphology of the polymer nanofibers. The SEM images of polyacrylonitrile only and polymer containing 0.5 g of moringa leaf extracts are shown in Figure 2. The SEM image shows that the morphology of the polymer nanofibers is cylindrical, smooth, and evenly distributed. The morphology remains almost the same after the incorporation of different concentrations of the leaf extract (SEM images of other concentrations not shown). It was observed that as the concentration of the leaf extract increases the viscosity of the

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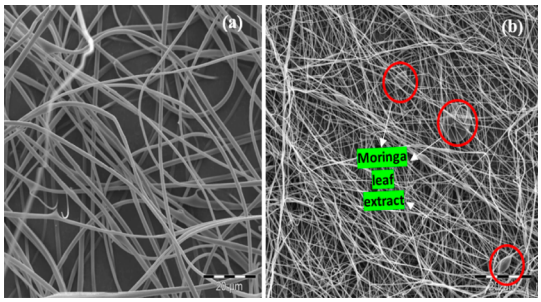

Figure 2. SEM morphology of 16 wt % (a) polyacrylonitrile only and (b) polyacrylonitrile with 0.5 g moringa leaf extract.

electrospinnable solution decreases, which gives rise to beaded nanofibers as seen in Figure 2b.[56]

2.1.1. Thermal Analysis on Nanofibers. Thermogravimetry was employed in order to determine the weight loss pattern of the polyacrylonitrile and the composite fibers as a function of temperature. Figure 3 shows the TGA curves of pure

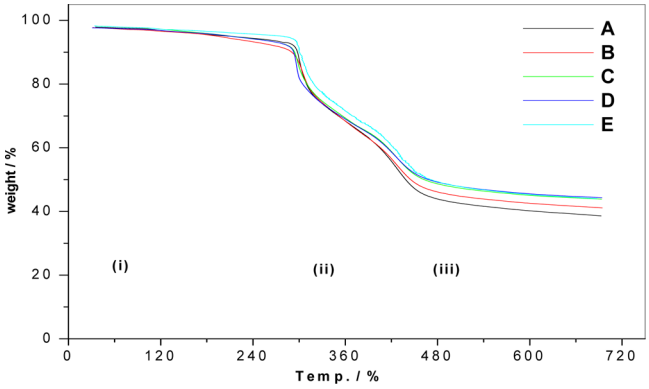

Figure 3. The TGA graphs of the 16 wt % polyacrylonitrile nanofibers (A) only and polymer with (B) 0.1, (C) 0.15, (D) 0.2, and (E) 0.5 g moringa extract.

polyacrylonitrile and the polymer nanofibers at different percentage loadings. About three-step degradation processes have been identified, which are characterized by an exothermic reaction typical of polyacrylonitrile for both the pure polymer nanofibers and polymer nanofibers with different concentrations of moringa extracts.[57] The three-stage degradation process involves the following: (i) the formation of rings among adjacent CN functional groups in the polyacrylonitrile structure. This process is reported to increase the thermal stability of the compound[58] and (ii) the decomposition of the rest fiber. In this stage, the effect of the addition of the moringa leaf extract becomes conspicuous as a slight temperature difference between the polyacrylonitrile and its composite is observed. In the third stage (iii), the residue left after the thermal decomposition showed increments which varied linearly with the percentage of moringa extract loading. The thermal stability of the nanocomposites showed improvement in the high-temperature region even with the lowest moringa extract concentration. This indicates a good interaction between polyacrylonitrile and the extract, thus resulting in the enhancement in thermal stability.

2.2. Wound Healing Studies. In the wound healing study, skin incisions on the rats dorsal with an area of 0.8 cm^2 were treated using a commercial antibacterial gauze (positive control, tagged F), nanofibers (negative control, tagged A), and nanofibers having different concentrations of moringa extracts incorporated (tagged B, C, D, and E). The % wound closure at 1, 4, 7, and 10 days of treatment is presented in Figure 4. The histology results (Figure 4) revealed that all groups showed improved healing with the wounded skin being completely re-epithelialized at day 11.

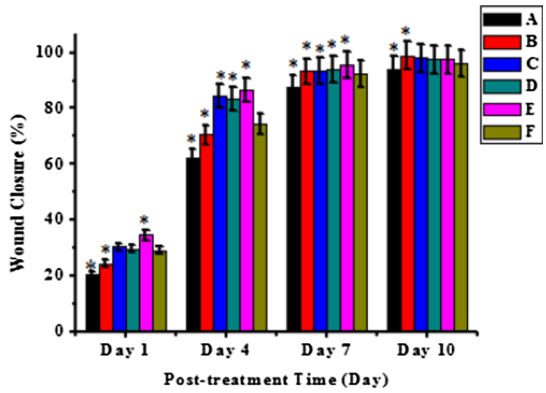

Figure 4. Wound closure (%) at 1, 4, 7, and 10 days after treatment (A–F, where A is polyacrylonitrile nanofibers only, B–E are polymer nanofibers with 0.1, 0.15, 0.2, and 0.5 g moringa extracts, respectively, and F is the positive control). The data are expressed as mean ± standard deviation from six independent experiments. *Statistically significant ($p < 0.05$).

At day 1, among the rats treated with the nanofibers, E (group E) showed the highest % wound closure among all other rat groups. This was even higher than those treated with the commercial antibacterial gauze, F. This trend was also observed at days 4 and 7. Interestingly, among the group treated with the negative control nanofibers, A also showed some wound closure property but not as much as those treated with nanofibers with moringa extracts in groups B–E. This is consistent with other reported literature.[15,49] The percentage wound closure for all the groups A–F increased gradually as the treatment time increases. For days 1, 4, and 7, the % wound closure was the highest for group E (35, 87, and 95% respectively) compared to the positive control at group F (29, 75, and 93% respectively) and other groups ($p < 0.05$). At day 10, group B showed the highest wound closure though not significant to groups C, D, and E. Group A showed the lowest wound closure compared with control and other groups ($p < 0.05$).

Figure 5 shows the histological images of the wounded skin from different groups. The images for that of groups A–E showed a concentration-dependent healing process as group E showed the best wound healing ability with complete re-epithelialization as well as almost completely healed wound, and almost intact arrangement of collagenous fibers in the dermis. As the concentration of the moringa extract content in the nanofibers increased, there is a corresponding increase in the % wound closure. This could be attributed to the antioxidant, anti-inflammatory,[36] and antimicrobial[37] properties of the moringa extract which improved the wound healing process of the injured rats. The result also showed that group A (which is the group treated with only nanofibers) had the least healing process observed.

2.3. Antibacterial Studies. The antimicrobial properties of *M. oleifera* plant extracts have been extensively studied and

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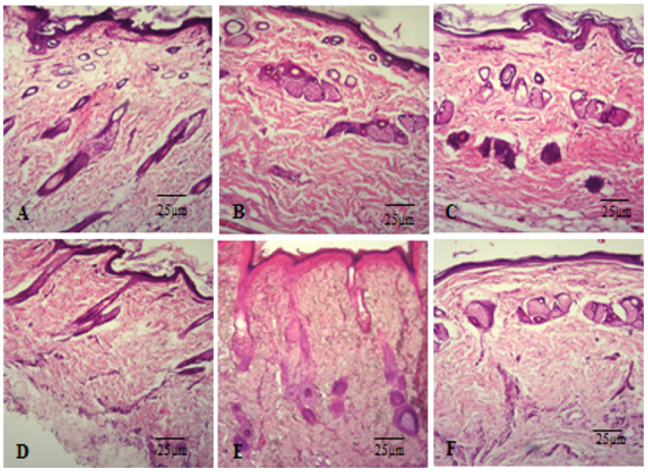

Figure 5. Histological images of the wound skins at 11 days after the treatment with different wound dressing materials A–F, where A is polyacrylonitrile nanofibers only, B–E are polymer nanofibers with 0.1, 0.15, 0.2, and 0.5 g moringa extracts, respectively, and F is the positive control.

reported.[36,37] *M. oleifera* extracts also have a potent inhibitory effect against multidrug-resistant methicillin-resistant *S. aureus*.[59] The polyacrylonitrile nanofiber mats (containing 0, 0.1, 0.15, 0.2 and 0.5 g of *M. oleifera* and 16 wt % polymer) were evaluated for their antibacterial properties against *S. aureus* and *E. coli*. The nanofiber mats loaded with *M. oleifera* extracts (B–E) exhibited concentration-dependent antibacterial activity against *S. aureus* and *E. coli* as shown in Table 1 and Figure 6. The nanofiber mat (E) loaded with 0.5 g of *M. oleifera* extracts exhibited the best antibacterial property compared to the other nanofibers. The nanofiber mat (A) without *M. oleifera* extracts incorporated had the least antibacterial activity. The results indicated that the *M. oleifera* extracts still retained its antibacterial activity, and even enhanced activities was observed despite been processed by electrospinning; as a consequence, nanofibers B–E exhibited antibacterial activities.

Table 1. Antibacterial Activity of 16 wt % Polyacrylonitrile and Polymer Nanofibers Containing Different Concentrations of Moringa Leaf Extracts[a]

compounds	*E. coli* (mm)	*S. aureus* (mm)
A	R	R
B	5 ± 1.4	3 ± 0.7
C	9 ± 2.1	6 ± 0.7
D	12 ± 0.7	7 ± 1.4
E	15 ± 0.7	12 ± 0.0
F	13 ± 1.4	7 ± 0.7
streptomycin	20 ± 0.0	23 ± 0.0

[a]A is polyacrylonitrile nanofibers only, B–E are polymer nanofibers with 0.1, 0.15, 0.2, and 0.5 g moringa extracts, respectively, and F is the positive control.

The results of the wound healing experiment were in agreement with other results in the literature as shown in Table 2.

3. CONCLUSIONS

In the present study, different concentrations of moringa extracts were incorporated into polyacrylonitrile nanofiber mats using the electrospinning process. The fiber mats provided suitable, simple, and cost-effective materials that were used in wound healing and antibacterial experiments. The wound healing experiment showed that nanofibers with different concentrations of moringa extracts (groups B–E) gave better wound healing properties compared to the usage of only the nanofiber (group A) because of the presence of the leaf extract with active functional groups. It also showed that the healing properties of the nanofibers (B–E) were a function of treatment time and concentration of the moringa extract as there was gradual skin re-epithelialization as the treatment days and concentration of the moringa extract increased, respectively. The nanofiber with 16 wt % of polyacrylonitrile and 0.5 g of moringa extract (E) gave the best wound healing property which was even better than the commercial gauze (Steri-tulle, Yangzhou, CN). Similarly, the antibacterial studies showed that *S. aureus* and *E. coli* were susceptible to nanofibers with incorporated moringa extracts (groups B–E). The antibacterial results also reflected concentration-dependent activities as increase in the concentration of *M. oleifera* extracts loaded in the nanofibers resulted in increased antibacterial activities. These antibacterial electrospun nanofiber mats have promising potential for use as effective wound dressings.

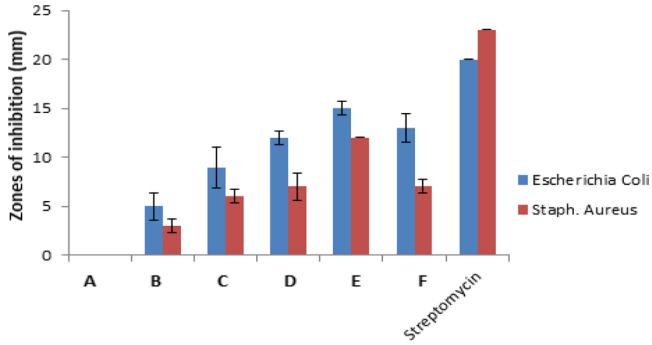

Figure 6. A histogram representation of the antibacterial sensitivity of the substances, where A is polyacrylonitrile nanofibers only, B–E are polymer nanofibers with 0.1, 0.15, 0.2, and 0.5 g moringa extracts, respectively, and F is the positive control.

4. EXPERIMENTAL SECTION

4.1. Materials for Preparation and Characterization of Fabricated Nanofibers. Polyacrylonitrile, moringa leaf, ethanol, dimethyl formaldehyde, and other reagents used were of analytical grade. Sixty Wistar rats were acquired from Animal house of the College of Basic Medical Sciences, Cross River State University of Science and Technology, Nigeria, including reagents such as ketamine hydrochloride, diazepam, alcohol, iodine, aspirin, and distilled water. The fabricated nanofiber were characterized by using a FT-IR spectrometer attached to a PerkinElmer Auto Image Microscope System equipped with a liquid nitrogen cooled MCT detector; morphological images were obtained by using field emission scanning electron microscopy purchased from JEOL JSM 5800 LV (Japan), and the thermal stability of the nanomaterials were determined by using the TGA.

4.2. Fabrication of Polyacrylonitrile Nanofibers Containing Moringa Leaf Extract Nanofibers. The nanofibers were fabricated by dissolving different concentrations of moringa leaf extracts into 16 wt % polyacrylonitrile. Each products were labeled A–E, where A is polyacrylonitrile nanofiber only amd B–E are nanofibers containing 16 wt % polymer and 0.1, 0.15, 0.25 and 0.5 g moringa extract,

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Table 2. Antibacterial Activity of Selected Nanofibers Containing Plant Extract Component[a]

plant extract	polymer for nanofiber matrix	microorganism	inhibition zone diameter (mm)	application	reference
green tea	chitosan−PEO	*E. coli*	4.0	wound dressing	60
		S. aureus	6.0		
chamomile	PCL−PVP	*E. coli*	7.6	wound dressing	61
		S. aureus	7.6		
geraniol	PVA	*E. coli*		food industry and cosmetics	62
		S. aureus			
moringa leaf extract	PAN	*E. coli*	15.0	wound dressing	this work
		S. aureus	12.0		

[a]PAN: polyacrylonitrile, PVA: polyvinylalcohol, PCL−PVP: poly(ε-caprolactone)/poly(vinyl pyrrolidone), and PEO: polyethylene oxide.

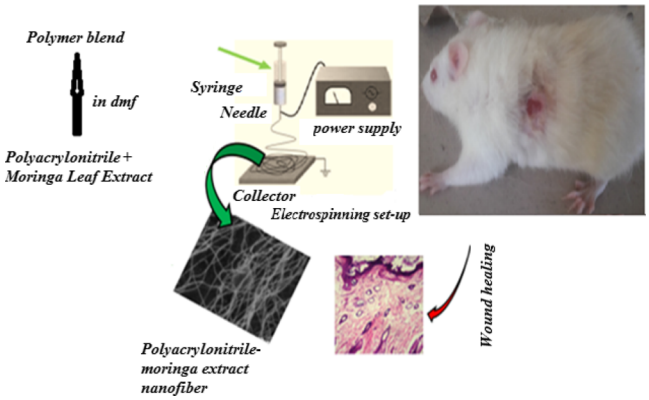
respectively. The polymer solutions were taken into 20 mL syringe with the needle and the tip of the needle being connected to a high voltage power supply of 20 kV, and nanofibers were collected on the aluminum foil collector plate at a distance of 10 cm from the needle. Electrospinning was performed at room temperature at a feed rate of 0.15 mL/h. The removal of the solvent and water from the electrospun samples was carried out by drying the nanofibers at room temperature for 24 h.

4.3. Wound Healing Experiment. The experiment involved 36 healthy Wistar rats with weights between the range of 150−200 g according to the methods reported in literature.[51] The Wistar rats were allowed to familiarize with their new environment for a week and were fed on standard rat pellets and water ad libitum. The assembling of the rats was done in six groups, namely, A, B, C, D, E and F, with six rats in each group and housed in individual compartment of plastic cages. The caring guidelines given in NIH for rats and laboratory animals were strictly followed in the handling of the rats used for this study.[52]

4.3.1. Induction of Skin Lesion on the Animals. The animals used for the study had no pre-existing skin lesion. Before the commencement of the surgical proceedings, the animals were weighed individually and recorded. The anesthetics used were 10% ketamine hydrochloride (Rotex-medica, 0.1 mL/kg body weight) and diazepam (0.1 mL/kg body weight) and were administered intramuscularly. An imaginary line was drawn from the shavened right dorsolateral aspect of the thoracic wall of the animals from the lower margin of the ear. The area was cleaned with antisepsis. In the center of the shaved area, a surgical skin lesion of 0.8 cm by 0.8 cm area of the skin was measured and excised by exposing the dorsal muscle fascia with the aid of a surgical scalpel. Care was taken to remove the panniculus carnosus. 100 mg/kg weight of aspirin was given to the rats for pain control.

4.3.1.1. Procedures for Wound Dressing. Scheme 1 represents the wound dressing procedures used for this work. Rats in groups A, B, C, D, and E had their wounds dressed with nanofiber patches, whereas the wound of rats in group F was dressed with a commercial gauze (Steri-tulle, Yangzhou, CN) which also served as our positive control. The nanofibers used for group A rats contained only 16 wt % polyacrylonitrile nanofibers with no moringa extract which was used as the negative control. The nanofibers used for groups B to E rats contained 16 wt % polymer and 0.1, 0.15, 0.25 and 0.5 g moringa extract, respectively. The nanofibers and the commercial gauze were secured over the wounds of the animals as shown in Scheme 1. The dressing of the wound was carried out every 2 days. The area of the wound was measured

Scheme 1. Schematic Diagram of Nanofiber Application for Wound Healing



every 2 days. The percentage of wound closure was determined by using eq 1.

$$\text{Wound closure}(\%) = \left(1 - \frac{A}{A_i} \times 100\right) \quad (1)$$

where A_i is the initial wound area and A is the wound area after a fixed time interval.

The capturing of the images of the skin wounds for the whole process in the study was done by using a digital camera (14.1 megapixels) at a distance of 15 cm. The photos of the wound surface were imported onto ImageJ software. The pictures were calibrated in millimeters with the aid of a rule included into the image taken. The area around the wound surface were drawn with a freehand tool on ImageJ and measured in mm^2.

4.4. Histological Examination. The rats used for the study were sacrificed at the 11th day under the use of ether anesthesia. 10% buffered formalin was used to preserve and store the specimens of the wound area. Slices of 5 μm were marked with hematoxylin−eosin for the demonstration of skin architecture of the rats, and Masson trichrome was used for the demonstration of collagen fibers. The inspection of these sections was carried out by using the Leica DM750 microscope with a digital camera attached. Digital photomicrographs of the tissue sections were taken at 100× magnification.

4.5. Antibacterial Activity. The antibacterial sensitivity of the nanofibers (A−E) to *S. aureus* (*S. aureus*) and *E. coli* (*E. coli*) using the agar diffusion method. The choice of the bacterial organisms was due to their relevance to wound infections. The Petri discs contained 20 mL of sterile Mueller−Hinton agar. The bacterial suspension of approximately 1×10^6 cfu/mL was swabbed on the solidified agar media and left to

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dry for 15 min. Thereafter, 5 mg of the nanofibers was placed on each disc. The loaded discs were left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 h at 37 °C and closely monitored for the development of clear zones around the nanofibers. The antibacterial sensitivity was judged by the diameter of the zone of inhibition in millimeters. The experiment was repeated twice, and 1 mg/mL streptomycin was used as a positive control. The experiment was done twice, and the antibacterial results treated with statistical software SPSS 21.0. The antibacterial study was done according to the methods reported in the literature.[53]

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Author Contributions

O.E.F., A.C.E. and L.K.-S. designed the work. O.E.F. carried out the fabrication of the materials and the characterization. D.C.O. also contributed to the characterization of the fabricated nanomaterials. A.C.E., A.P.E. and O.M.I. carried out the antibacterial and wound dressing applications. E.E.E. contributed to interpreting some of the data generated, editing, and finalizing the manuscript. All the authors contributed to the writing and proofreading of the manuscript.

Notes

The authors declare no competing financial interest.

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