Original Article

Leprosy-associated Chronic Wound Management Using Biomaterials

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Abstract

Background: Deformities and neuropathic chronic ulcers are the common features associated with leprosy-cured individuals that impact their quality of life and impair rehabilitation efforts. The challenging aspects for treatment of chronic wounds are the factors that inhibit healing. We reasoned that limited success of various therapeutic interventions could be due to the fact that leprosy-cured individual's physiology gets acclimatized to having a chronic wound that any therapeutic intervention is counterbalanced to maintain *status quo* at the wound site. Therefore, an alternative strategy would be to use biomaterials that gradually alter the wound site allowing the individual's physiology to participate in the healing process. **Aims:** Developing the human amnion (Amn)-derived biomaterial scaffolds and evaluating its use to heal chronic wounds in leprosy-cured but deformed persons (LCDPs). **Materials and Methods:** Using an enzymatic protocol, we have developed a rapid method to generate biomaterial scaffolds from discarded human Amn. A clinical trial on 26 LCDPs was performed with the biomaterial, and its wound-healing potential was then compared with LCDPs undergoing standard treatment procedure. **Results:** Biomaterial-based treatment of chronic wounds on LCDP displayed a higher efficiency in healing when compared to standard treatment. **Conclusions:** This study exemplifies that biomaterial-based treatment of leprosy-wounds offers an excellent affordable alternative for wound management. This study underlines the importance of involving both local wound environment and systemic effects for healing. In addition, we highlight wound healing as a necessity for successful rehabilitation and reintegration of leprosy-cured person into the society.

Keywords: Amnion, biomaterial, chronic, leprosy, wound

Introduction

Leprosy, a treatable, genetically nontransferrable disease caused by *Mycobacterium leprae,* neuropathic chronic ulcers, and deformation of the affected region, are a common sequelae.[1] Posttreatment for *M. leprae* infection, leprosy-cured but deformed persons (LCDPs) who are otherwise considered disease-free and healthy harbor disabling chronic wounds.[1] Chronic wounds are usually characterized by the presence of high level of inflammatory cells, elevated protease activity, defective extracellular matrix (ECM), and failure of epithelialization that prevents healing. In addition, clinical impediments, such as diabetes, hypoxia, inflammation, infections, and patient's health status, are other factors that contribute to delay in healing. These studies indicate that both local wound environment and systemic effects should be sought in case of impaired healing.[2] We reasoned that similar to physiological acclimatization to chemical, biological, and environmental stressors, acclimatization to chronic wound might also be a reason inhibiting healing in LCDPs. Therefore, any direct therapeutic intervention directed toward the wound without taking into account the individual's physiological adaptation to the wound might impede healing. Indeed, reports discussing immune systems adaptation to local microenvironment and modulation of adaptive immunity to achieve better results for biomaterial-driven tissue regeneration are a testament to the importance of physiological modulation for faster healing.[3] Therefore, a gradual change instead of

Access this article online

Quick Response Code:

Website: www.jgid.org

DOI: 10.4103/jgid.jgid_79_17

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How to cite this article: Sivasubramanian S, Mohana S, Maheswari P, Victoria V, Thangam R, Mahalingam J, *et al*. Leprosy-associated chronic wound management using biomaterials. J Global Infect Dis 2018;10:99-107.

direct intervention to alter the local wound environment allows individuals physiology to adapt and facilitate healing. This might in part explain the limited success of therapeutic strategies, such as use of recombinant growth factors, inflammatory response modulators, steroids, vitamins, and cell therapy on the wound site.[4-10]

Biomaterials offer a good platform to orchestrate such gradual change at the wound site.[11] Various biomaterials have already been tried as wound dressing, for delivery of drugs, growth factors, or cell therapy with varying degree of success.[12,13] Clinical trials with amniotic membrane showed encouraging results, but the labor and time-intensive mechanical separation procedure to isolate collagen-rich amnion (Amn) and the antigenicity of amniotic epithelium and chorion (Cho) are limiting factors.[14] The aim of the current study was to develop human Amn-derived biomaterial scaffolds using an enzymatic separation protocol and evaluate its efficacy in healing chronic wounds in LCDP.

Here, we report biomaterial-based modulation of chronic wound site in LCDPs. We use a simple enzymatic separation method to process human Amn into biomaterial scaffolds and perform the clinical evaluation. This work demonstrates that these biomaterials offer an alternative and affordable strategy for wound healing in leprosy-cured individuals. We also demonstrate that this uncomplicated way to modulate local wound environment is enough to close chronic wounds that were unresponsive to standard antiseptic wound treatments. In addition, we demonstrate the pliant nature of these materials for affordable prosthesis development and highlight the necessity of wound management for successful rehabilitation of LCDPs into the society.

Materials and Methods

Standard care and ethical permits

Human placenta collection, study protocol, and subject consent for clinical trials on LCDPs having wounds were overseen by the Central and State Government Committee Members along with approved Institutional Ethical Committee (IEC number Ref: KIPMR Letter – 002/DIR/RESEARCH/KIPMR dt 05/09/2013, CSIR-CLRI Letter – D/8/2013 dt 15/08/2013, BCG Vaccine Laboratory Letter – D. O. No. F. 20016/4/2013 Admn. Dt. 05/09/2013) constituted by the King Institute for Preventive Medicine and Research and Central Leather Research Institute, India. Human placenta was collected post delivery from mothers who were tested negative for various infectious agents, including toxoplasmosis, HIV, syphilis, measles, rubella, cytomegalovirus, and herpes simplex virus and hepatitis viruses. The absence of these agents in placenta was confirmed at King Institute of Preventive Medicine and Research which is a state government referral laboratory for viral diseases. A single-site randomized wound healing study was carried out at rehabilitation center of Sri Ramakrishna Math for leprosy-cured persons, located in Chennai, India. Leprosy-cured persons who did not respond to normal antiseptic wound dressing for a minimum of 2 months were eligible for this study. Patients were recruited using informed consent forms in both English and the local language before the commencement of the study; confidentiality was maintained regarding the participant's identity. The trial was conducted in compliance with applicable regulatory requirements in accordance with the provisions of the Declaration of Helsinki and in adherence to good clinical practice.

Human placenta processing and preparation of Amn membrane

Human fresh placentas, weighing about 500–800 g, were obtained by normal healthy delivery from women without a history of premature rupture of membranes and were moved to processing unit. Blood clots and debris of the umbilical cord were completely removed through rinse with saline and extensive wash with 0.1 M phosphate-buffered saline (PBS), followed by dipping them in 100% w/v of PBS bath containing 1000 casein digestion units of a bacterial protease (for 1 kg of placenta) lacking collagenase and elastase activity from *Bacillus subtilis* MTCC 5333 (a patented enzyme; Indian Patent No. 271983) at 30°C for 30 min. The protease activity is expressed in terms of tyrosine equivalents using Hammarsten casein. To 1.9 ml of 1% casein solution prepared in 0.1 M carbonate buffer of pH 9.5, 0.1 ml of suitably diluted enzyme solution is added and the reaction mixture is kept at 40°C for 10 min. The reaction is terminated by the addition of 3 ml of 5% trichloroacetic acid solution. The absorbance of the trichloroacetic acid soluble filtrate at 280 nm is measured and one unit of enzyme activity is defined as the liberation of 1 μM tyrosine equivalent of substrate per ml or per gram of enzyme/min (casein digesting unit [CDU] = release of 1 μM tyrosine from casein substrate/ml or g/min). The bath was drained off, and the detached amniotic membranes from Cho and decidua of placenta were recovered (Indian Patent Application No. 3049/CHE/2012). These membranes were further subjected to enzymatic treatment in a bath of 100% w/v of Amn containing the said protease having 1500 CDU per kg of Amn for 90 min and 3 h to obtain epithelium-free Amn membrane and to extract collagen from the recovered Amn, respectively. The membranes thus obtained were taken for further processing for therapeutic purposes. All unused biological materials were discarded as biowaste, and standard guidelines of biowaste handling were followed.

Biomaterial sterilization

Amn material was used in both preclinical and clinical studies. Postenzymatic separation, Amn materials were washed extensively with 0.1 M PBS-containing antimicrobial chemicals such as 25–50 μM each of amphotericin B, gentamicin, chlorhexidine, and metronidazole and immersed in the said antimicrobial solution for 30 min. Postchemical disinfection, the membrane was spread onto the filter paper and kept in the Biosafety Level II laminar hood and subjected to ultraviolet (UV) sterilization for 30 min. Post-UV sterilization, the material was radiosterilized using 25 kGy ^{60}Co gamma radiation.

Patient recruitment and grouping

LCDPs with plantar wounds who had not responded to normal Eusol antiseptic wound dressings or had no prior reported sensitivity to collagen were eligible to participate in the biomaterial-based healing study. In addition, LCDPs in this study were (i) leprosy-cured male and female patients aged ≥18 years; (ii) presence of chronic leg ulcers, further defined as follows: (a) leg ulcer with CEAP classification of C6 with duration <24 months and (b) wound area range between 4 cm^2 and 15 cm^2 (as measured by the greatest length multiplied by greatest width); (iii) patient's leg ulcer should meet at least three of the five signs such as pain between two dressing changes, perilesional skin erythema, edema, foul odor, and heavy exudation; and (iv) patients/legal representatives who are able and willing to sign informed written consent. Patients who have malignant wounds or had recent deep venous thrombosis or venous surgery; progressive neoplastic lesion treated by radiotherapy or chemotherapy and on-going treatment with immunosuppressive agents or high dose of corticosteroids; poor nutritional status in the opinion of investigator; smoking or alcohol addiction; and psychiatric condition or drug abuse problems were excluded from the study. Patients requiring partial or complete amputation; patients treated with drug(s) that, in the opinion of the investigators, will affect the study objectives or patient safety and pregnant and lactating mothers were also excluded from the study.

A total of 26 patients were included in the study after screening. Out of 26, 14 patients permitted taking photography and Bates-Jensen Wound Assessment Tool (BWAT) analysis. The protocols for informed consent forms in English and the local language were provided. They were educated about the necessity to maintain physical hygiene and wound care aspects during the study period. Randomization was done based on wound size at the time of screening. Wound size was grouped into small, medium, and severe, and the patients were assigned to two treatment groups based on their health status. Treatment Group 1 consisted of individuals who were leprosy-cured, disease-free and healthy, and treatment Group 2 consisted of patients who were leprosy-cured and disease-free but had other health conditions such as blood pressure, heart ailments, and diabetes [Supplementary Table 1]. The biomaterial-based evaluation was compared to control group study consisting of eight LCDPs who were administered standard antiseptic wound dressing for wound management. All patients in this study had ambulatory nature of occupation and their wounds were characterized with pus and other exudates associated with chronic wounds. Before initiation of therapy, details of leprosy-cured persons such as demographic details, nature of occupation, wound details including history, duration, location, size, and area (length, breadth, and depth), magnitude (small, medium, and large), number of wounds, discharge or release of exudate from wounds, pain at the time of dressing, perilesional skin erythema, edema, signs of infection (increased pain, increased redness, wound drainage), bleeding, change in wound color and/or odor, irritation (increased redness and/or inflammation), maceration (skin whitening) and hypergranulation (excessive tissue formation), and medical history including other ailments and deformity details were recorded with the support of medical experts. General health aspects of the subjects during the study were monitored by physicians every week. Raw BWAT scoring and individual photographs of control and bio-material treatment photographs can be obtained from the authors.

Patient treatment protocol

The ulcer wounds were debrided, cleaned, and dressed with Amn scaffold (treatment group) or Edinburgh University Solution of Lime (Eusol) dressing according to the World Health Organization (WHO) guidelines (control group). New Amn scaffolds were applied to the wound site upon complete digestion of older material into the wound. At each dressing change, wounds were inspected and cleaned exclusively with normal saline. If necessary, mechanical debridement was performed to remove slough and necrotic tissues. The Amn material was cut to suit the size of the wound, placed on the wound surface, padded with gauze, and covered with light bandage. Amn treatment was continued till complete closure of the wounds was observed. The control treatment was carried out till no visible improvements to wound closure were seen. The control treatment was stopped when further treatment did not result in any more healing.

The subjects were assessed for sensitivity (allergic reaction), signs of infection or pain if any, extent of wound healing, etc. during every change of dressing. Review of patients' filled-in diary cards for the assessment of compliance, adverse events, and concomitant medications was performed during the study. If wound closure was achieved, subjects were relieved from the study and end of treatment procedure was administered.

Statistics and outcome documentation

Medical assessment of wounds before initiation of therapy and extent of wound healing during and after completion of therapy was carried out by BWAT. The scores were made independently by clinical experts who were blinded to the study. Thirteen parameters describing the wound status in the subjects were assessed by BWAT through visual analog scale (1–5 scoring method). Low and high scores indicate the extent of regeneration and degeneration, respectively. Baseline score of 13 indicates healthy nature of tissue.

Efficacy parameters such as wound size reduction from baseline (absolute and relative), number of dressings required to achieve wound closure with granulation and epithelialization, wound closure rate, change of local signs at dressing change interval, local adverse events, dressing acceptability, and subjects' response and cooperation during this study were documented.

Foot prosthesis development

To generate footwear, the affected foot was photographed and scanned using Kinect 3D scanner and a three-dimensional (3D) model was generated using 3D builder software. In parallel,

the unaffected foot was used to make a mold, scanned, flipped, and overlayed over the affected foot model to match the size and shape. Once this was done, footwear was printed using professional grade silicone rubber and polyurethane casting resin using imakerobots.com services. Postprinting, the footwear was allowed to cure, lined inside with biomaterial, spray painted outside to match patients skin tone, and glued onto commercially available footwear.

Cell culture

Human foreskin-derived fibroblasts were cultured on Amn placed in DMEM supplemented with 10% fetal bovine serum (FBS) and 1X penicillin-streptomycin (Pen-Strep) (all from Invitrogen). Postculture, confluent cells were split 1:3–1:5 using TrypLE Express (Invitrogen). Cells were dissociated with trypsinization (TrypLE E™ Express 1X, Gibco).

Tensile and tear tests

The automatic control electronic universal testing machine (UTM, H10KS, Tinius Olsen) according to the ASTM D 638-03 method was used to measure tensile parameters. Specimen length and diameter were measured using a reading microscope. The test was performed to determine the capability of a material to resist the deformation during stretched. Barrier properties (oxygen transmission rate) of the samples were characterized by Noselab Ats. The prepared films were determined on samples cutting into small pieces (2 cm × 3 cm). The samples were first dried in a vacuum drier at 60°C for 2 days. The WVTR of the Kc, Amn and Amn-Kc films was calculated by Mocon Permatran according to the standard of ASTM F 1249-90. Five samples were prepared and the average values were calculated. Specimens from the normal human amniotic membrane group were 25 mm long and 9.8–10.2 mm in diameter. Each specimen was preset by 10 repeated loading and unloading. The experimental temperature was close to normal human body temperature (36.5 ± testing machine, with a loading speed of 5 mm/min). To maintain humidity in the specimens, a liquid spray was continuously administered. On experimental completion, the following indices were automatically generated from the automatic control electronic UTM: maximum load, maximum displacement, maximum stress, maximum strain, elastic limit load, elastic limit stress, and stress–strain curve 1.0°C.

Water uptake capacity

Measured unit of dried scaffolds were weighed and placed in a watch glass filled with deionized water and retained for 20 min. Difference in the weight was measured before and after placing in watch glass filled with water to find out the actual water uptake capacity.

Scanning electron microscopy

The scaffolds with fibroblasts, after 96-h culture, were removed carefully and fixed with 1.5% glutaraldehyde in PBS for 15 min at room temperature, washed three times with PBS, and dehydrated stepwise with ethanol. For scanning electron microscopy (SEM) analysis, after critical point drying, the samples were gold coated under vacuum and viewed with a JEOL 8401 scanning electron microscope.

Cell viability assay

To assess the effect of Amn on cell viability, the sheets were cut into circular patches to fit into a well of a 96-well plate. Fibroblast cells grown to 80% confluency were taken, trypsinized, and plated into a 96-well plate at a density of 3000 cells per well. The plate was incubated overnight with DMEM, 10% FBS, Pen-Strep media, following incubation circular Amn membrane were cut and presoaked in DMEM for 20 min and were added on top on the cells in 96-well plate. The plate was incubated for 2 days and cell viability was assessed using CellTiter-Glo kit according to the manufacturer's protocol.

Results

Collagen-rich Amn biomaterial was prepared from human amniotic membrane using a new enzymatic protocol [Figure 1a]. The amniotic membrane was isolated from human placenta and subjected to processing using one-step enzymatic treatment to effortlessly peel different layers of Amn. The collagen-rich Amn region devoid of epithelial layer and Cho was processed into Amn scaffolds [Figure 1a]. Histological examination of the preprocessed amniotic membrane showed an epithelium layer [Figure 1b black arrowhead], collagen-rich Amn [Figure 1b red arrowhead], and Cho [Figure 1b asterisk], and topological examination using SEM indicated a rough Amn part [Figure 1c represented as Amn] and smooth Cho part [Figure 1c represented as Cho]. Histological analysis of postprocessed Amn that the collagen-rich Amn part could be isolated with or without the epithelial layer [Figure 1d i-ii]. Enzymatic processing did not cause deterioration of membrane histology, elastin, or polysaccharide content [Figure 1d iii-v]. SEM of postprocessed Amn membrane displayed a rough topology with approximately 6 μm thick columns packed together [Figure 1e i] similar to that seen with the preprocessed material [Figure 1c]. In addition [Figure 1d], longitudinal and transverse sections of the Amn material showed the columns to be long and hollow and regularly spaced [Figure 1e iii-iv]. These analyses indicated that enzymatic processing completely gets rid of Cho while retaining the original architecture of the Amn layer. Amn scaffold therefore could be molded into various configurations and its hollow columns acting as an adsorbent to imbibe exudates from the wound site or be loaded with drugs.

Amn biomaterial could be used in the generation of large sheets [Figure 1f] which could then be effectively used as scaffolds for culturing fibroblast cells. Fibroblast cells grown on Amn scaffolds showed increased cell viability [Figure 1g]. Materials also showed a strong tensile strength, oxygen and water vapor permeation, and elongation capacity [Figure 1a]. These results indicate that the biomaterials actively promote cell viability. Given that fibroblast cells are very important players in the process of wound healing,[15] materials that promote its viability will have an advantageous effect on

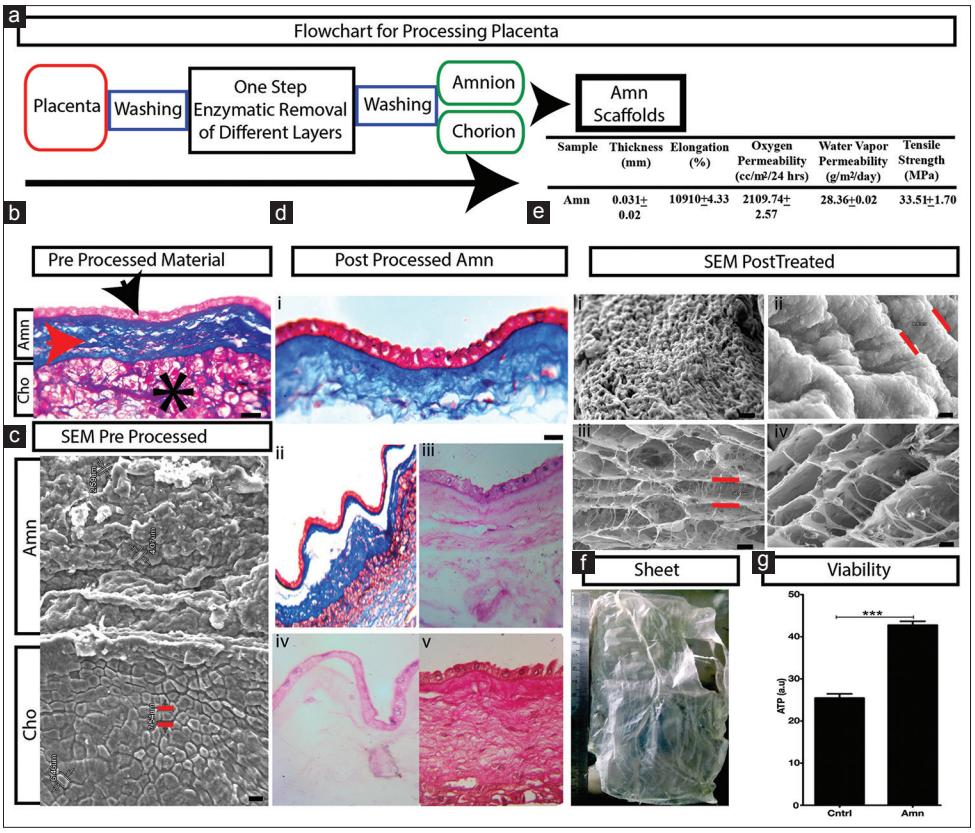

Figure 1: Biomaterial development and characterization. (a) Flowchart representation of enzymatic processing of placenta and the observed biophysical features of the processed amnion scaffolds. (b) Masson's Trichrome staining of preprocessed amnion material showing amnion (red arrowhead) and chorion (asterisk) layers with collagen stained in deep blue, epithelial cell layer (black arrowhead) of amnion and lipid content of chorion stained in pink. (c) Scanning electron microscopy of preprocessed amnion showing a lipid-filled chorion that is clearly demarcated from collagen-filled amnion layer. (d) Histology staining of postprocessed amnion membrane, (i, ii) Masson's Trichrome staining of collagen-rich part of the amnion membrane with or without attached epithelial layer. Collagen is stained in deep blue, (iii) Verhoeff's elastic staining on amnion membrane showing cationic, anionic, and nonionic bonds with elastin in pink, (iv) Hematoxylin and eosin staining of postprocessed membrane showing nucleus of the epithelial cell layer of amnion shown in blue, rest of the tissue is in pink color, (v) Periodic acid-Schiff staining showing polysaccharide content of the postprocessed amnion membrane. (e) Scanning electron microscopy on posttreated amnion showing a multigroove structure (i) that is regularly spaced (ii) having hollow interior (iii, iv). (f) Postprocessed amnion processed as a thin sheet. (g) Cell viability measurement of fibroblast grown without (Control) or without amnion scaffold

increasing the healing efficiency. Various growth factors, such as epidermal growth factor, fibroblast growth factor, and transforming growth factor, have been extensively used to increase fibroblast cell proliferation and to promote chronic wound healing with varying degree of success.[16] Growth factor therapy has encountered various roadblocks such as decreased site availability due to higher protease activity at wound site, scarring and activation of other signaling cascades, and therapy cost and duration.[16] In addition, these growth factors have also been linked to epithelial malignancies making them less attractive for long-term therapeutic application.[17] These biomaterials with their beneficial effect on cell viability offer a very good alternative to growth factor-based therapy without having the same side effects. These results indicate that the observed physical properties of biomaterials can stabilize wound structure, act as an absorbent gradually removing exudate, proteases, and other secretary inflammatory factors, reduce microbial growth, avert drying, allow oxygen and water vapor permeation, is absorbed at wound site, promotes healing and wound closure. In addition, they facilitate fibroblast cell viability to promote wound healing.

To test whether this approach can be applied to chronic wounds on leprosy-cured persons, a clinical trial was carried out on individuals who were harboring chronic wounds for more than 5 years, showing no response to normal antiseptic wound dressing and lacking collagen sensitivity [Figure 2a]. The aim was to explore whether Amn biomaterial can offer an affordable alternative for wound healing. A total of 26 individuals were selected showing a median age of 64 years (equal number of males and females) [Supplementary Table 1]. Patients were classified into two groups: LCDP but healthy individuals as Group 1 and Group 2 were LCDP but with other health complications [Figure 2a,c and Supplementary Table 1], and wounds were classified as small, medium, or severe [Figure 2a,c and Supplementary Table 1]. Treatment was done using freshly processed Amn material that was sterilized

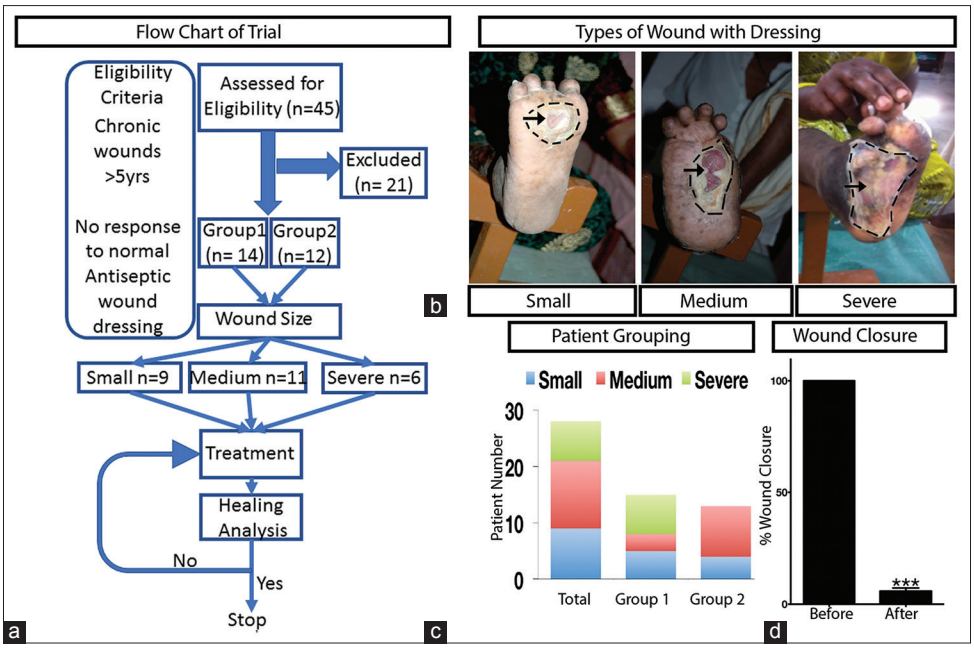

Figure 2: Clinical trial preparation. (a) Flowchart indicating clinical trial design. (b) LCDP photographs showing wound (black arrow) and different wound categories treated with biomaterial dressing (black dashed lines). (c) Graphs showing total number of patients taken for clinical trial with grouping of them based on wound categories (small, medium, and severe) and health status (Group 1 or Group 2). (d) Average of the total percent area of wound closed postamnion treatment

and applied topically on the wound site [Figure 2b]. Topical application with biomaterials enhanced healing resulting in 95% closure in all the patients with complete closure seen in 80% of the patients irrespective of the group they belong [Figure 2d and Supplementary Table 1]. Treatment with Amn material quickly decreased pus and exudate secretion in addition to removal of severe odor from the wound site [Supplementary Table 1]. The material got gradually digested and absorbed completely. On the other hand, Eusol treatment (control group) did not produce complete healing and lasted for an average of 4 months with an average of 16 dressing changes. Control trials were stopped after no significant improvement to wound was seen [Figure 3a]. Every patient differed with the number of dressing required to achieve wound closure. In LCDPs treated with Amn, small category wound required an average of 9.8 dressings per person with the minimum of 5 and maximum of 18 dressing changes. Medium category wounds required an average of 10.7 dressings per person with a minimum of 4 and a maximum of 18 dressing changes. Severe category wounds required an average of 14 dressings per person with a minimum of 4 and a maximum of 20 dressing changes [Supplementary Table 1]. There was no dramatic difference seen between the healing efficiency or number of dressing required for wound closure between patients from Group 1 or 2 category [Supplementary Table 1]. These results indicate that performing biomaterial-based simple alterations to local wound site is enough to promote healing of chronic wounds in LCDPs. Amn directed local wound alterations such as stabilization of wound structure; removal of exudate while keeping the wound moist and aerated enhances the wound-healing capacity of LCDPs from Group 1 and Group 2 category [Supplementary Table 1].

Amn treatment produced a gradual and visible healing of wounds causing closure of small, medium and severe wounds when compared to that of control groups [Figure 3a]. The healing efficiency was subjected to multiparametric assessment using BWAT. The BWAT analysis displayed a significant improvement in healing in the biomaterial-based treatment group when compared to standard control treatment [Figure 3b]. In addition to employing biomaterials for chronic wound healing, we explored the use of Amn for reconstructive prosthetic development. This would offer a path toward improving the psychological state of the LCDPs and aid in their social acceptability. To facilitate this purpose, a prototype was developed by preparing plaster-molding of a foot model [Figure 3c i]. The developed foot model was layered inside with biomaterials and coated externally with silicone [Figure 3c ii-iii]. The printed prosthetics was stuck to available footwear for patient use [Figure 3c iii]. These results suggest that these biomaterials can be used for prosthetics development and can aid in the rehabilitation efforts. These results also demonstrate that use of Amn biomaterial offers an affordable, efficient, and uncomplicated way to manipulate wound site. This approach promotes healing of chronic wounds of LCDPs and is amenable to prosthetic development.

Discussion

Rehabilitation effort of LCDP individuals is very complicated. Social stigma, cultural understanding, economic status,

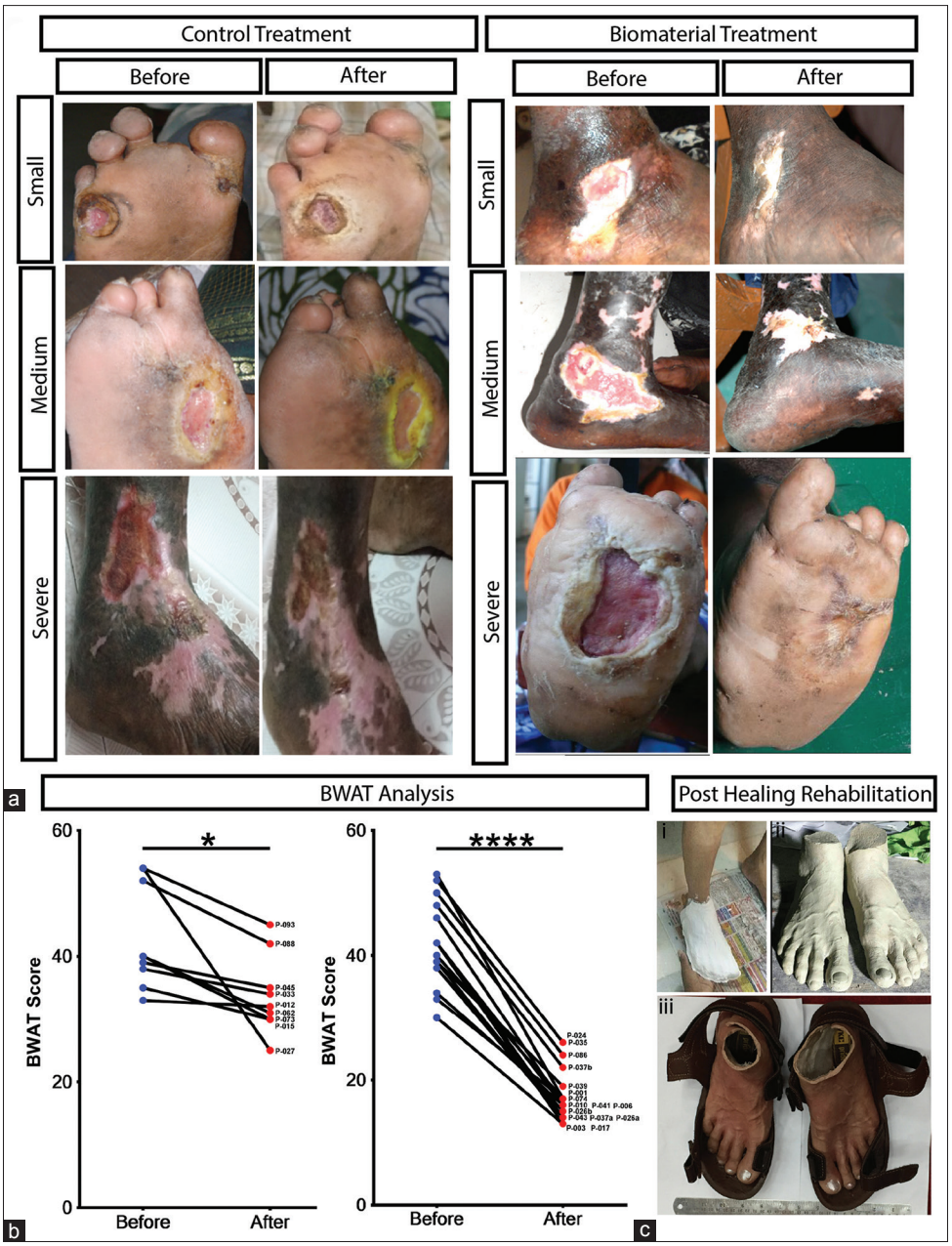

Figure 3: Clinical trial on leprosy-cured individuals. (a) Before and after treatment photographs of different wound categories in the control and biomaterial treatment groups. (b) Bates-Jensen Wound Assessment Tool-based evaluation of chronic wound pretreatment (shown in blue) and posttreatment (in red) labeled with patient ID. (c) Posthealing rehabilitation program for patients showing normal foot molding (i), symmetrical footwear development (ii), and coloring of footwear and attachment to sandals for use (iii)

individual's psychological state, visible deformities, and chronic wound are the challenges that have to be overcome to facilitate successful rehabilitation.[18,19]

Chronic wounds in LCDPs offer a unique setup to evaluate approaches for wound healing. These individuals who are otherwise disease-free harbor debilitating wounds that hamper their quality of life, rehabilitation efforts, and result in increased healthcare burden. In addition, the lower economic status experienced by most of these individuals limits access to advanced therapies for wound healing and management. This highlights the need for affordable alternatives for wound care that can deliver similar results. Collagen-rich human Amn is the innermost region of placenta and has a demonstrated applicability for wound healing.[14,20] It is a readily available resource that is discarded postchildbirth and can be repurposed as an affordable alternative for wound healing in LCDPs. However, processing of Amn is labor and time intensive due to employment of mechanical peeling procedure. The enzymatic separation described here overcomes these difficulties and therefore could be adapted for scale up production and processing of Amn biomaterial. Collagen is widely accepted as a safe and multifunctional material which can also serve

as substrate for the excess proteases from the wound site, thereby decreasing protease-mediated impediment to wound healing.[21-24] This enzymatic procedure can be altered so as to obtain epithelial layer with or without collagen-rich basement membrane. Therefore, this procedure can be easily applied to generate materials for a wide variety of clinical scenarios including corneal repair, reconstruction procedures, burns injury, and other type of chronic wounds.

The rough topology and hollow columns of the Amn material as seen with the electron microscopy analysis indicates that the material can adhere to the wound and act as an absorbent of all the exudates, excess proteases, and debris at the wound site. The removal of impediments facilitates healing process to proceed, thus allowing ECM generation at wound site and promoting healing. The excess protease allows for digestion and absorption of Amn material at wound site, thereby avoiding manual removal of the biomaterial. This digestion process also limits the effect of Amn material to be transient, thereby giving the body more control over healing process and tissue remodeling.

Fibroblasts cells play a very important role in wound healing; they secrete new ECM and collagen structures on which other cells take support and perform effective healing.[25] Decreased viability and phenotypic alterations of fibroblast are generally seen in chronic wound situation. This causes delayed or slow healing process and contributes to chronic wound condition.[26] The Amn biomaterial used here effectively stimulates fibroblast viability, thereby indicating that the material not only acts as a scaffold to fibroblast cells but also allows them to be viable. This characteristic feature is important to allow fibroblast to repopulate wound site to promote healing. Since the material is digested at the wound site, the effect on fibroblast remains transient, thereby allowing individuals healed skin to effectively regulate healing and tissue remodeling.

Chronic wound management places an enormous burden on healthcare system and has to be dealt by controlling both local wound environment and systemic effects.[27] The ease for preparing these materials provides an affordable alternative to existing therapies and their biophysical characterization suggests that the material permits modulation of the local wound environment and by that its systemic effects. Tensile strength of these materials reinforces local structure, while permeability to oxygen and water vapor allows the wound to prevent hypoxia and desiccation, factors important in chronic wound healing.[28-32] Thin width, rough topology, and transparent nature allow easy application of the material and visual monitoring of wound site. These results demonstrate that Amn biomaterial offers an affordable, practical, and an uncomplicated way for modulating wound environment. Although we have evaluated the performance of Amn material in comparison to Eusol treatment, we have not compared it to growth factor-based therapies that are also available for chronic wound management. However, this method of gradual alteration to wound site is more desirable to direct intervention therapies, such as use of growth factors, as it provides an opportunity for individual's physiology to slowly adapt to the changes at the wound site. In addition, Amn biomaterial avoids the challenges and malignancies that are often associated with growth factor and other interventional therapies.

Conclusions

Chronic wounds are usually characterized by the presence of various clinical impediments.[5-7,9,27,33,34] Local and systemic effects produced due to slow evolution; prolong incubation, and neurological impairment of leprosy lead to chronic wounds. Individuals continue to harbor wounds leading to a debilitating condition that delays recovery and restricts rehabilitation placing an enormous burden on the healthcare system.[35] In this report, we demonstrate that by harnessing favorable physicochemical properties of biomaterials, we can gradually alter wound site which promotes healing. Promoting healing via systemic effects produced through modulating local wound environment might be an appropriate means to engage individual's physiology to participate in healing. The uncomplicated nature of this approach and higher degree of healing of wounds strongly advocate its application in recovery and rehabilitation efforts of LCDPs. In addition, they offer a cheaper alternative to existing therapy such as use of growth factors, which have been widely associated with various epithelial malignancies.[36] The clinical results indicate its potential application for chronic wounds, in healthy LCDPs (Group I) and LCDPs with secondary complications (Group 2). The pliant nature of these materials permits its use in wound healing and prosthesis development, thereby aiding in patient recovery and rehabilitation efforts. The current study also encourages testing of these materials in other types of chronic wound conditions.

Acknowledgements

The authors greatly acknowledge Sri Ramakrishna Math, Chennai, India for permitting and facilitating this study as a part of the rehabilitative services for empowering LCDPs.

Financial support and sponsorship

SK would like to thank Lillian Sagens och Curt Ericssons Forskningsstiftelse and Vetenskapsrådet for funding. RT would like to thank postdoctoral project DST SERB (PDF/2016/000086) for postdoctoral funding support.

Conflicts of interest

There are no conflicts of interest.

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