Nanofabricated Wound Matrices and Mechanisms of Action

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PRIMARY AIM: Perform a systematic review of scientific publications related to nanofiber wound matrix materials and underlying mechanisms of action

Introduction: Wound matrix materials are used to improve the regeneration of dermal and epidermal layers in both acute and chronic wounds. Contemporary wound matrices are primarily composed of biologic materials such as processed xenogeneic and allogeneic tissues. Unfortunately, existing biologic wound matrices possess multiple limitations including poor longevity, durability, strength, and enzymatic resistance required for persistent support for new tissue formation. A fully-synthetic, resorbable nanofiber material (Restrata Wound Matrix, Acera, St.Louis, Missouri) that exhibits structural similarities to the native extracellular matrix offers a new approach to the treatment of acute and chronic wounds.

Methods: The present study consists of a systematic review of scientific publications related to wound matrix material technologies and underlying mechanisms of action. This review of the material technologies that currently exist to address chronic wounds aimed to discuss what makes for an optimal wound matrix and introduce a novel synthetic nanofabricated wound matrix. The present study was also designed in order to outline the unique mechanism of action underlying novel nanofabricated wound matrices.

Results: The nanofiber wound matrix is made from synthetic biocompatible materials and was designed to include a fibrous structure with high porosity, similar to native ECM. The electrospun material is a porous matrix with a defined rate of resorption that provides a scaffold for cellular infiltration and vascularization before completely degrading via hydrolysis. The device permits the ingress of cells and soft tissue formation in the defect space/wound bed. The device does not contain any human or animal materials or tissues. The fibers are produced using a fully validated commercial scale electrospinning process. Thus, fiber elements whose scale and topography resemble native ECM are achieved with high reproducibility. The architecture, surface topography, and structural scale of wound matrices have a significant effect on the biological activity of regenerative cell populations. The matrix possesses a fully-synthetic nanoscale architecture that enables unique material and biologic properties ideally suited to wound healing scenarios. These unique attributes of the nanofiber matrix are intended to support wound healing by encouraging tissue regeneration, neovascularization, and epithelialization.

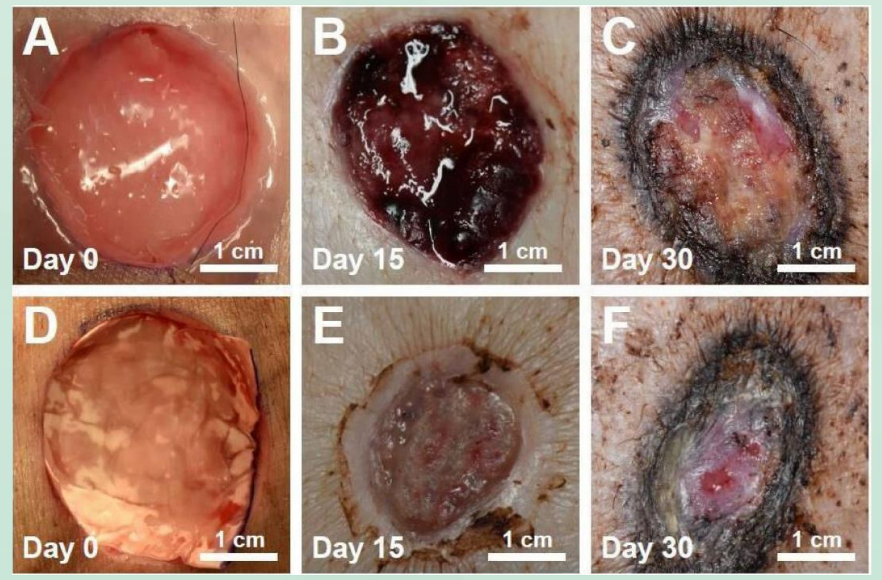
Conclusions: The fully-synthetic, resorbable nanofiber material that possesses structural elements similar to natural ECM, offers a new approach to the treatment of acute and chronic wounds by combining the advantages of synthetic construction with the positive attributes of biologic materials. Further characterization of the novel nanofabricated material in pre-clinical and clinical settings demonstrate the breadth of clinical utility.

BACKGROUND: Fully-Synthetic Nanofiber Wound Matrix Mimics Human ECM and Supports Cell Ingrowth, Retention, and Differentiation

- Restrata™ Wound Matrix is the only persistent, flexible, fully-synthetic, bioengineered skin substitute for use in wound closure
- Restrata™ is composed of non-woven, resorbable synthetic nanofibers whose structure and architecture mimics that of native ECM
- Due to Restrata™'s fully synthetic design, the matrix resists enzymatic degradation, persists in the wound bed, reduces inflammatory response, and supports cellular/tissue ingrowth
- The unique properties of Restrata™ also offers improved ease-of-use, flexibility, and clinical versatility with significant logistical advantages over existing amniotic / allogenic products

- Restrata™ exhibits structural attributes similar to native extracellular matrix
- Electrospun nanofibers support cellular ingrowth, retention, and differentiation while directing and enhancing cellular activity
- The porosity and progressive resorbtion of Restrata™ supports continued cellular infiltration, tissue formation, and neo-vascularization
- Prior studies confirm nanofiber materials are a unique alternative to allografts / xenografts

Restrata™ Facilitated Significantly Greater Speed and Quality of Wound Healing Compared to Integra™ Wound Matrix[12]



METHODS: Systematic Review of Peer-Reviewed Literature Related to Nanofabricated Materials

A systematic review of peer-reviewed publications related to nanofiber wound matrices was performed

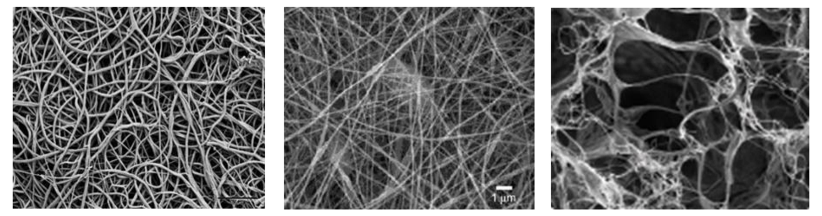
Underlying mechanisms of action were identified

- **Similar structure and architecture to human ECM**
- **Supports cellular ingrowth, differentiation, and retention**
- **Supports cellular infiltration and neovascularization**
- **Fully synthetic construction offers excellent biocompatibility**
- **Fully synthetic construction offers resistance to enzymatic degradation**
- **Persistence in the wound bed may lead to fewer reapplications**

RESULTS: Nanofiber Wound Matrices Possess Unique Mechanisms of Actions that Support Wound Healing by Encouraging Tissue Regeneration, Neovascularization, and Epithelialization[13]

Nanofiber Wound Matrix has a Structure and Architecture Similar to Human Extracellular Matrix

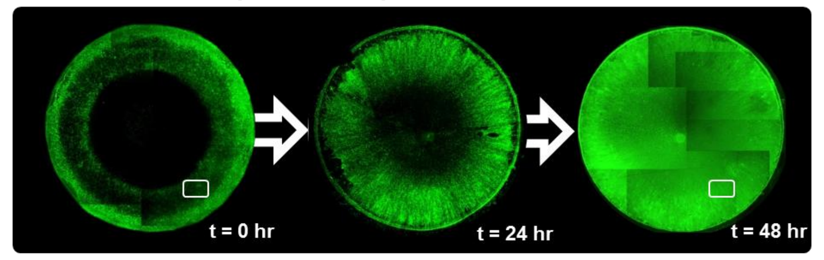
The nanofiber wound matrix constructed of a blend of electrospun synthetic nanofibers that are similar to the structure of native ECM. In native skin, ECM is composed of non-woven collagen fibers approximately 50-500 nm in diameter [1]. In electrospun materials, the size of the polymer fibers can be tuned by parameters such as polymer concentration, polymer flow rate, and applied voltage [2]. Electrospinning, therefore, allows for the reproducible production of fibers that simulate the size and organization of natural ECM fibers [3]. The RWM is composed of non-woven synthetic fibers with a mean fiber diameter < 2000 nm and therefore mirrors the fiber size and organization of native ECM. Due to the unique biomimetic architecture, nonwoven electrospun materials similar to RWM have been successfully used in the engineering of bone, skin, and blood vessels [2].



Nanofibers Support Cellular Ingrowth, Differentiation, and Retention

Electrospun nanofibers have been shown to support cellular ingrowth and migration critical to successful wound healing. Fibroblasts have been shown to proliferate and spread more rapidly and upregulate collagen expression on PLGA fibers 350-1100 nm in diameter, as compared to fibers of smaller or larger dimensions [4,5]. Cell spreading and migration have also been influenced by the diameter of electrospun fibers. Synthetic fibers 350-1100 nm in diameter have been shown to affect the spreading, migration, and phenotype of adherent fibroblasts and mesenchymal stem cells (MSCs) [5,6]. The electrospun nanofiber matrix's non-woven synthetic fibers are ideally positioned in this range of fiber diameters in order to support cellular migration into the matrix. Therefore, the unique blend of synthetic electrospun nano- and micro-fibers may support both rapid cellular ingrowth, migration, and activation, as well as continued cell retention and proliferation.

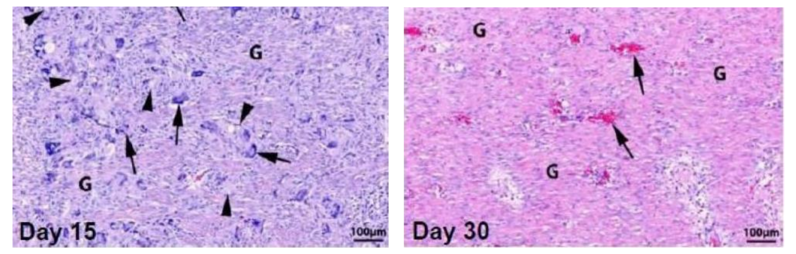
Nanofiber material promotes rapid cellular infiltration and activation



Nanofiber Matrix Supports Cellular Infiltration and Neovascularization

Nanofiber wound matrices have engineered porosity suitable cellular migration and tissue ingrowth as well as transport of oxygen, nutrients, and waste [2]. The high surface area afforded by the nanoscale fibers provides a substantial substrate for cell attachment, oxygen permeation, and the management of exudate [4,6,7]. The pore size and degree of porosity are also critical in facilitating cell migration and vascularization [5,6]. Highly porous matrices (60-90% porous) are advantageous for cellular infiltration and tissue ingrowth, while pore sizes of 90-130 μm are sufficient to permit fibroblast migration and proliferation [8,9]. Pore sizes of 5-500 μm diameter have also been shown to facilitate the successful invasion of new vasculature into the construct's interior, the absence of which contributes to cell death and tissue necrosis [5]. The porosity of non-woven synthetic fiber matrix is ideally positioned within this range in order to support cellular ingrowth and neovascularization.

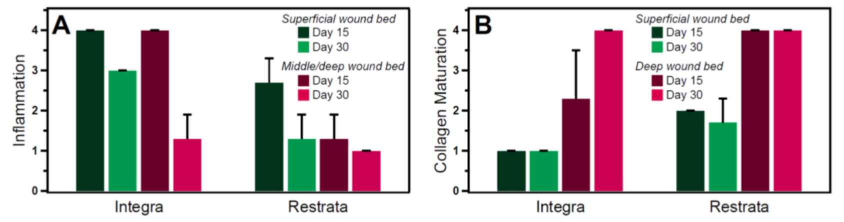
Nanofiber matrix supports tissue ingrowth and neovascularization



Fully Synthetic Construction Offers Excellent Biocompatibility

The synthetic composition of the nanofiber matrix offers a material devoid of any risk of allergic response to animal products, zoonotic disease transmission, or ethical/religious concerns. The use of well-characterized medical grade synthetic polymers that exhibit excellent biocompatibility can also significantly reduce the inflammatory response at the site of application. Compared to the inevitable host response elicited by biological materials from foreign human (allograft) or animal (xenograft) sources, electrospun wound matrix materials may offer reduced levels of inflammation within the wound bed both at acute and sub-acute timepoints. The nanofiber matrix is also fully resorbable, similar to leading biologic xenograft and allograft products. The resorbable nature of the electrospun matrix ensures that no permanent implant or material will remain at the site of application and that no chronic inflammatory reaction against the material will exist to limit wound healing or resolution.

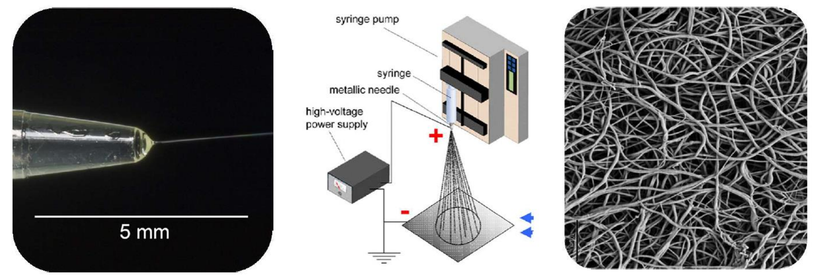
Nanofiber matrix exhibits lower inflammation than collagen matrix[12]



Fully Synthetic Construction Offers Resistance to Enzymatic Degradation

Resorption of electrospun polymer matrix occurs in controlled stages that are governed by progressive hydrolysis rather than rapid enzymatic proteolysis. The nanofiber matrix is composed of multiple electrospun polyglactin 910 and polydioxanone fiber populations. The synthetic electrospun fibers used to create the nanofiber wound matrix are therefore resistant to key enzymes released by inflammatory cells (e.g. MMPs) within the wound bed. The nanofiber matrix thereby resists rapid enzymatic degradation at the wound site, avoiding premature degradation by proteases that directly contribute to and are overexpressed in chronic non-healing wounds. Wound matrices such as electrospun nanofiber matrix that can resist protease degradation can provide a more stable and a more persistent scaffold capable of supporting wound healing over a longer time course [10].

Nanofiber matrix constructed of fully-synthetic resorbable polymers



Increased Persistence in the Wound Bed May Reduce Reapplication Rates

The nanofiber wound matrix has been engineered to resorb at a rate that matches the time course of tissue ingrowth. Due to its polymeric composition, the matrix gradually resorbs via hydrolysis within the wound bed over the course of 2-4 weeks. This prolonged presence within the wound site is anticipated to provide continued support of cellular infiltration, vascularization, and reepithelialization, which occurs over a course of three weeks in normal wound healing [11]. The time-scale over which the nanofiber matrix is present within the wound bed is significantly longer than existing biologic scaffolds currently in the clinical use. The longevity of the nanofiber matrix within the wound site may enable a longer period of wound healing and cellular ingrowth, and possibly eliminate the need for continued reapplication over the course of treatment. Thus, the maintenance of the wound site may only require changing outer wound dressings, rather than the wound matrix, and reduce the number of painful procedures experienced by the patient. Furthermore, complete resorption of nanofiber wound matrix precludes any persistent chronic foreign body response.

Nanofiber matrix may require fewer reapplications in difficult wounds

CONCLUSIONS: Nanofiber Wound Matrices Possess Unique Mechanisms of Action

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