# Biomimetic Nanofibers Electrospun with Calreticulin as Wound Healing Agents

**Effective topical wound healing agent that stimulate tissue regeneration through diverse biological activities that correct multiple defects of the wound healing process for the treatment of chronic wounds, such as diabetic foot ulcers (DFUs) and to enhance the rate and quality of healing of acute wounds (burns, injury, surgery).**

### Unmet Need

Effective topical wound healing agents that stimulate tissue regeneration through diverse biological activities that correct multiple defects of the wound healing process for the treatment of chronic wounds, such as diabetic foot ulcers (DFUs) and to enhance the rate and quality of healing of acute wounds (burns, injury, surgery).

### Technology

The Gold lab, in collaboration with the Wang Biomedical Engineering Lab at Stevens Institute of Technology, has electrospun calreticulin (CALR), a protein that potently heals wounds by a tissue regenerative process, with polycaprolactone (PCL) and type 1 collagen (Col1) nanofibers (NFs). The team has found unexpected novel synergistic wound healing activities of CALR incorporated into NFs (**Figure 1**) particularly effective on primary fibroblasts derived from non-healing diabetic foot ulcers (DFUs). Previous *in vivo* studies (*Nanney et al., Am. J. Pathol 2008; Greives et al., Wound Repair Regen 2012*) demonstrated that topical CALR significantly enhances the rate and quality of wound healing in porcine and diabetic mouse excisional wounds. By day 10, CALR-treated wounds achieved complete re-epithelialization and a collagen-rich granulation tissue [neodermis], in contrast to wounds treated with Regranex (currently, the only FDA-approved wound healing agent), which lacked epithelization and had limited collagen-rich granulation tissue. *In vitro* studies using human wound healing cells (fibroblasts, keratinocytes, macrophages) show that the mechanisms of action of CALR (pM, nM levels) tissue repair are through stimulating proliferation, migration, extracellular matrix induction [e.g., collagen], and antimicrobial activity. A major defect of chronic wounds is a paucity of scaffold-like granulation tissue for the attraction of cells into the wound for reconstruction. Electrospun nanofibers containing natural fibers, such as collagen, provide scaffolds in which fiber architecture can be modified to elicit specific cellular behavior. The research team sought to employ PCL/Col1 NFs for prolonged delivery of CALR through a singular application, while also protecting it from environmental threats, such as nonphysiological pH conditions and high protease activity commonly found in chronic wounds. As published, (*Stack et al., ACS App Materials & Int 2022*), PCL/Col1 NFs electrospun with CALR [CALR-NFs or PCC NFs] retained all previously reported wound healing biological activities. Under physiological conditions [37°C in pH 7.4 (PBS)], favorable release kinetic studies with fluoresceinated CALR revealed a burst release of the protein within 4 hours, reaching a peak at 72 hours, and CALR-NFs had greater resistance to proteolytic degradation *in vitro* following enzymatic exposure. Uniquely, CALR-NFs induce

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synergistic responses by cells not achieved by CALR or NFs alone. Human fibroblasts grown on CALR-NFs are stimulated to proliferate and are phenotypically elongated, consistent with their enhanced rate of directed migration shown by time-lapse chromatography and an increased rate of migration in gap closure using an *in vitro* wound healing assay. Migratory ability was consistent with an increase in FAK phosphorylation at cell-substratum contacts, actin fiber formation, vinculin capping, and increased expression of integrin β1 and TGF-β1. In addition to these functions, keratinocytes show increased laminin-5 deposition and actin fiber polymerization. Furthermore, CALR-NFs activated macrophages to release cytokines and iNOS [for antimicrobial activity]. By RNAseq comparing primary fibroblasts derived from DFUs that healed and those that did not heal wounds [non-healer] within 12 weeks, 43 genes were upregulated by CALR-NFs compared to NFs alone, and importantly, non-healing fibroblasts were more highly responsive to CALR-NFs, showing cellular polarity, rapid migration in an *in vitro* wound healing assay, phosphorylation of FAK, TGF-β3, collagens I,IV, fibronectin, and MMP-2 synthesis. These results suggest that CALR-NFs have the unique potential to heal diabetic wounds most recalcitrant to complete and sustained healing by novel reprogramming of non-healer fibroblasts. The future of the wound healing field incorporates different scaffolds for treatment, but none have the broad-ranging and diverse biological activities of CALR that synergize with tunable natural [collagen] biomimetic NFs. The initial presence of the NF scaffold supplants the lack of provisional matrix in chronic wounds, essentially augmenting the tissue regeneration process for both acute and chronic wounds, such as DFUs.

## Background

Impaired wound healing is a major complication associated with diabetes mellitus (>30 million in the US) due to systemic hyperglycemia, causing biochemical alterations that affect the cellular constituents involved in wound healing. Chronic wounds, particularly diabetic foot ulcers (DFUs), present a significant clinical challenge and remain a global unmet medical need, imposing an annual cost of over $25 billion on the U.S. healthcare system. Approximately 30% of diabetic patients are expected to develop DFUs in their lifetime, yet conventional wound healing treatments for DFUs remain largely ineffective. DFUs exhibit a 65% recurrence rate within five years post-healing, a 15% amputation rate within the first year, and a five-year mortality rate of 50% among those who undergo amputation, underscoring the substantial burden placed on healthcare. Regranex, a gel that contains platelet-derived growth factor BB, is the only topical treatment for cutaneous wound repair approved by the FDA in the last 20 years; publications vary, showing limited to no efficacy. Additionally, various bioengineered skin substitutes (BES) available in the U.S. market are considered suboptimal. Considering the large number of rapidly increasing patients, due in part to obesity and the limited effectiveness of current treatments, the search for DFU cures has exploded, as evidenced by only 3 papers in 2008 to 100 in 2024. The field is moving towards encouraging intrinsic healing (stimulating cell responsiveness) using three-dimensional nanofibrous scaffolds composed of natural proteins such as collagen and fibrin. The CALR-NFs demonstrate the ability to address the urgent need for new therapeutic agents that can enhance wound healing through multiple mechanisms that directly address the numerous factors that impede the healing of chronic, poorly-healing, or non-healing wounds, such as DFUs.

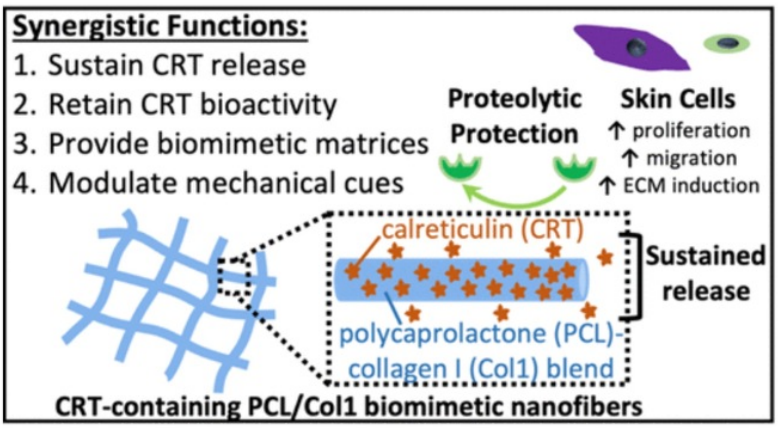


**Figure 1.** Schematic depicting the integration of CALR (CRT) into PCL/Col1 nanofibers, designed as biomimetic extracellular matrices, to provide an innovative long-lasting delivery method for CALR (CRT) with proteolytic protection, while enhancing its synergistic effects for tissue regeneration on cells in the wound bed.

### Applications

- Acute wounds, such as burns, injuries, or post-surgical wounds
- Poor to non-healing chronic wounds, such as diabetic foot ulcers and pressure ulcers (i.e., bed sores)

### Advantages

- **Enhanced protection:** NFs safeguard CALR from proteolytic and pH-unstable conditions common in chronic wounds.
- **Sustained CALR delivery:** Only one application of CALR-NF is necessary, reducing burden for both patients and clinicians.
- **Broad biological activity/cell responsiveness:** CALR-NFs stimulate the migration rate and proliferation of cells that heal wounds and enhance ECM protein synthesis. CALR-NFs activate macrophages and CALR mediates the innate immune response.
- **Synergistic effects:** Combination of CALR and biomimetic NFs provides an initial scaffold that recruits cells to the wound for reconstruction of the skin defect by stimulating cellular responses.
- **Specific effects on non-healing fibroblasts:** Unique potential for reprogramming fibroblasts from nonhealing DFUs to phenotypic and behavioral characteristics of normal wound healing fibroblasts.
- **Superior healing outcomes to Regranex:** CALR shows greater, wider-ranging and diverse cellular effects than Regranex, accounting for its superior healing effects.

### Intellectual Property

NYU has filed a co-owned, pending PCT patent application (with the Stevens Institute of Technology) that covers the composition of the polymeric matrix incorporating calreticulin, the method for producing this polymeric matrix, and its use in treating both acute and chronic wounds.

### References

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