JSNN · REU SUMMER RESEARCH

Macrophage-Targeted Nanomedicine in a Skin-on-a-Chip Model to Modulate Wound Inflammation and Reduce Fibrotic Scarring

Jessica Wei

Joint School of Nanoscience and Nanoengineering (JSNN)

PI: Zahra Sabet Mentor: Dr. Kerui Wu

The Problem We're Solving

WHAT THE DATA SHOW

44% of people with a scar report ongoing itching; 26% report pain

48.5% of adults surveyed internationally reported at least one scar

22% of recent scars were under a year old- an active, ongoing outcome

Source: see References, slide 13

CURRENT STANDARD OF CARE

- Pressure garments
- Topical silicone
- Corticosteroid injection

Each one acts on tissue that has already scarred, not on the biology that decided it would.

OUR APPROACH

Intervene earlier, during the immune response itself, so the scar-driving signal is dialed down before fibrosis begins.

Two Signatures, One Cell Type

Macrophages switch their entire secretory program depending on the signals around them — and fibroblasts read that program directly.

M1 — Classically Activated

Triggered by LPS / IFN-γ. Secretes TNF-α, IL-6, IL-12 to fight infection and remove debris.

M2 — Alternatively Activated

Triggered by IL-4 / IL-13. Secretes TGF-β, IL-10, VEGF to resolve inflammation and rebuild tissue.

What fibroblasts hear from M2

M2 paracrine signaling increases fibroblast proliferation and supports organized, load-bearing collagen remodeling.

What fibroblasts hear from M1

Sustained M1 paracrine signaling can raise fibroblast IL-6 and matrix-degrading MMPs by 10–100× — a profile linked to disorganized, excess matrix.

In short: fibroblasts don't scar on their own: they scar in response to what macrophages tell them.

Sources: see References, slide 13

Project Hypothesis

*We hypothesize that delivering **magnesium** (well known for its anti-inflammatory and pro-regenerative properties) directly to macrophages via a targeted nanomedicine during the early inflammatory phase of wound healing will promote macrophage polarization toward the M2 phenotype, thereby reducing pro-fibrotic signaling and improving tissue repair.*

If our nanomedicine reaches macrophages during the inflammatory window, we should be able to read that effect directly in their gene expression profile, even before any tissue-level scarring occurs.

SUCCESS CRITERIA

1. **M1 signaling drops**

   Lower pro-inflammatory output (e.g., TNF-α, IL-6) versus LPS alone

2. **M2 signaling rises**

   Higher pro-healing output (e.g., IL-10) versus LPS alone

3. **Fibrotic drive stays in check**

   No compensatory spike in collagen-related gene expression

Engineering the Model

A skin-on-a-chip device built from a 3D-printed mold, cast in PDMS, and populated with living cells.

1. Design & print the mold

Chip geometry designed in Fusion 360; master mold produced by stereolithography (SLA) 3D printing in a photocurable resin

2. Cast in PDMS

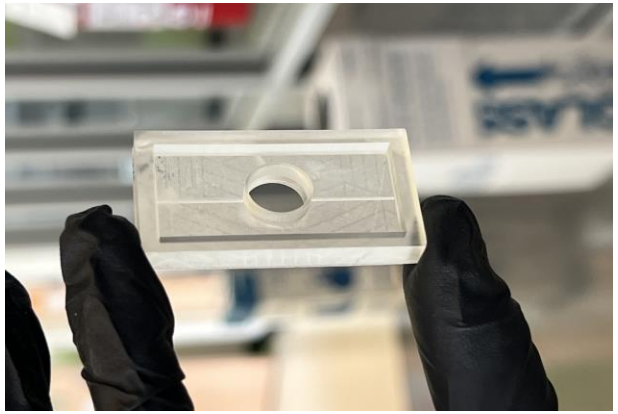
PDMS mixed at the manufacturer's base-to-curing-agent ratio, poured over the mold, degassed under vacuum, and cured; the cured replica is bonded to glass for a transparent, imageable device

3. Seed the co-culture

Macrophages and fibroblasts seeded into the chip

4. Run the system

Cells cultured, treated, and monitored over time



Finished PDMS chip — inlet port and channel

Master mold with cast PDMS chip

MEASURED DIMENSIONS

45 × 25 × 6.5 mm overall • Ø10 mm central opening • 4 mm chamber depth • 1 mm channel width

Characterizing the Nanomedicine

Before testing on cells, the magnesium-loaded nanoparticles were verified for size, shape, and elemental composition.

Dynamic light scattering (DLS)

Confirms particle size distribution is within the intended nanoscale range for cellular uptake

Transmission electron microscopy (TEM)

Visualizes particle morphology and confirms consistent, well-formed nanoparticle structure

Elemental mapping

Confirms magnesium (Mg^{2+}) is present and distributed within the nanoparticle structure

This characterization confirms the delivery vehicle itself — not just the downstream biology — behaves as designed before it ever reaches a macrophage.

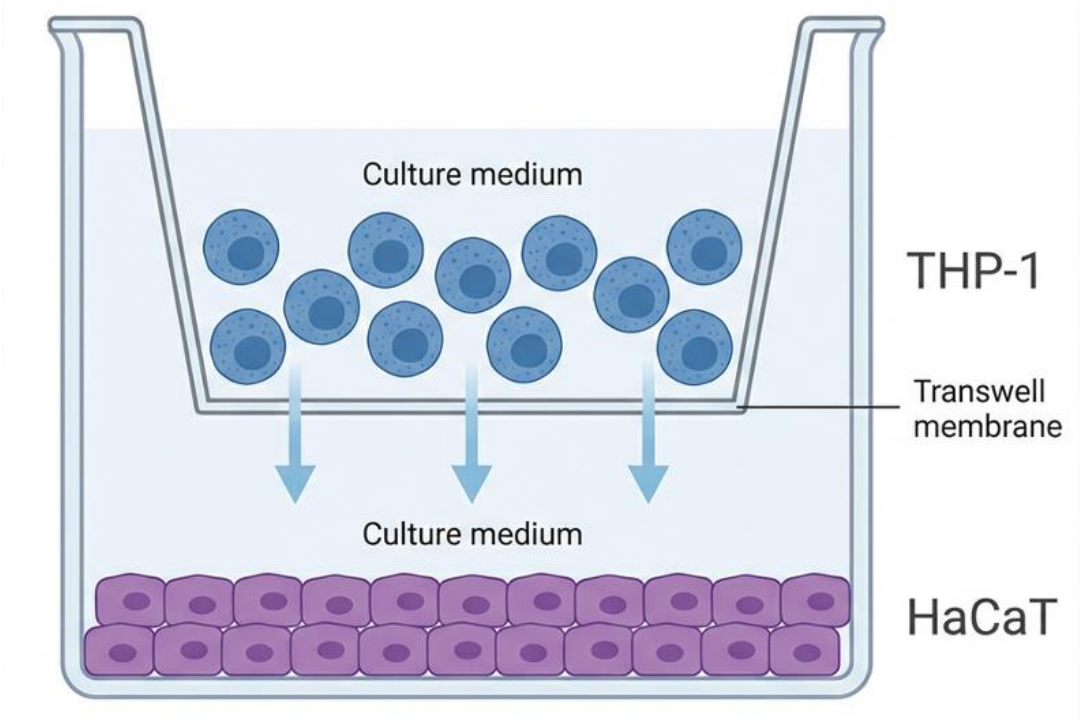


Fig. Dynamic light scattering (B) and TEM (C) of nanoparticles; element mapping of Mg^{2+} in nanoparticles (D).

Testing the Hypothesis

Three complementary cell models probe the same question at different levels of complexity.

THP-1 monoculture

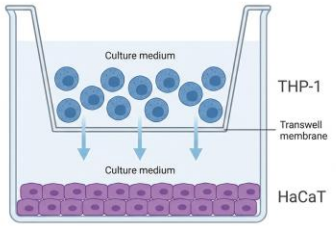
THP-1-derived macrophages alone: isolates the direct effect of the nanomedicine on macrophage gene expression

THP-1–HaCaT Transwell co-culture

Macrophages and HaCaT keratinocytes share media across a 0.4 µm porous membrane, no direct mixing, modeling paracrine crosstalk

HDF scratch-wound model

Human dermal fibroblasts (HDF) with an artificial scratch wound: tests the downstream fibroblast/fibrotic response



Transwell co-culture setup

THREE TREATMENT GROUPS

Control — baseline, no treatment applied

LPS — induces inflammation, modeling early wound response

LPS + Nanomedicine — tests whether the therapy shifts the inflammatory response

All groups are treated identically before comparison by RT-qPCR gene expression across all three models.

Lab Workflow

From cell preparation through gene expression analysis.

1. **Differentiate THP-1**

   PMA induces the macrophage phenotype

2. **Induce inflammation**

   LPS added to model wound inflammation

3. **Treat**

   Nanomedicine applied to designated groups

4. **Extract RNA / cDNA**

   RNA extracted and reverse-transcribed

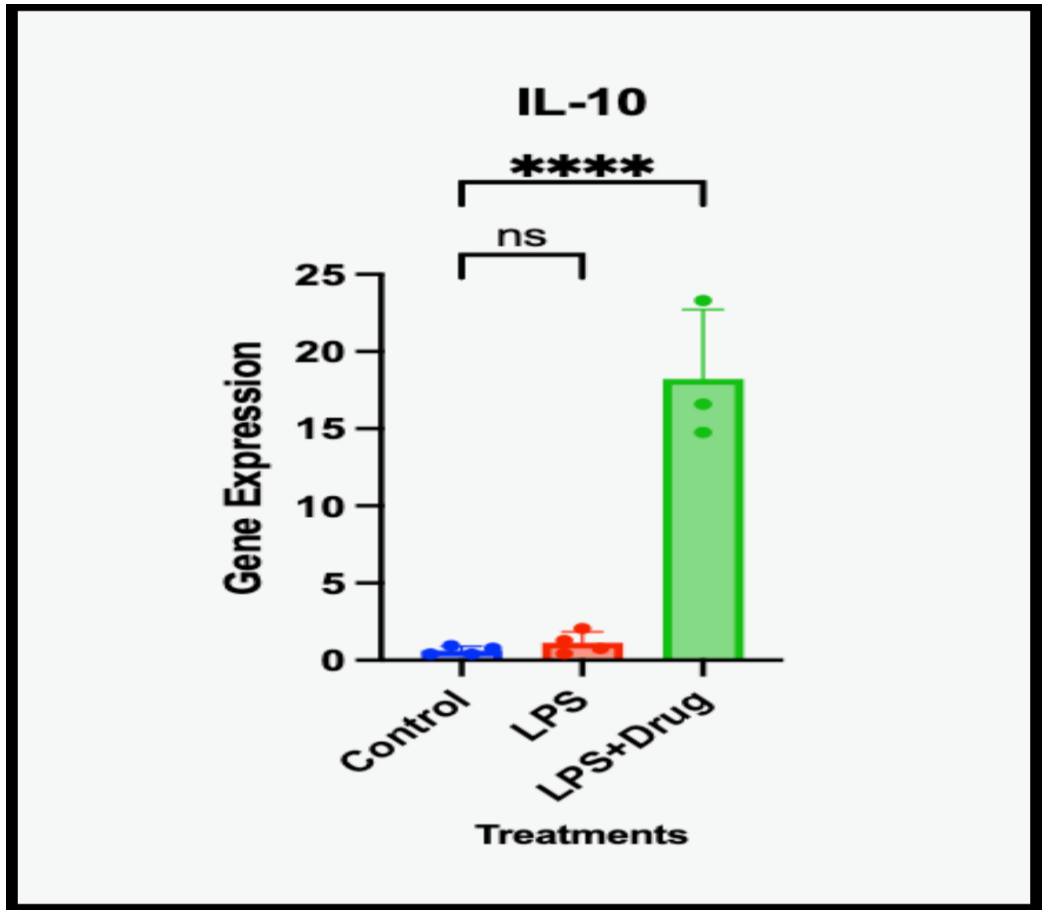
5. **RT-qPCR**

   Gene expression quantified across groups

Culture media prepared for HDF and THP-1

What We Found: IL-10 Expression

IL-10 is an anti-inflammatory, M2-associated marker of pro-healing macrophage activity.



p < 0.0001

LPS + Nanomedicine vs. Control and LPS alone

LPS alone

No significant change in IL-10 vs. control (ns)

LPS + Nanomedicine

Significant increase in IL-10 expression (**** p<0.0001)

Interpretation

Supports a shift toward the pro-healing M2 macrophage phenotype

What We Found: MMP7 & VEGFA

Beyond IL-10, two additional markers strengthen the case for a matrix-remodeling, repair-associated response.

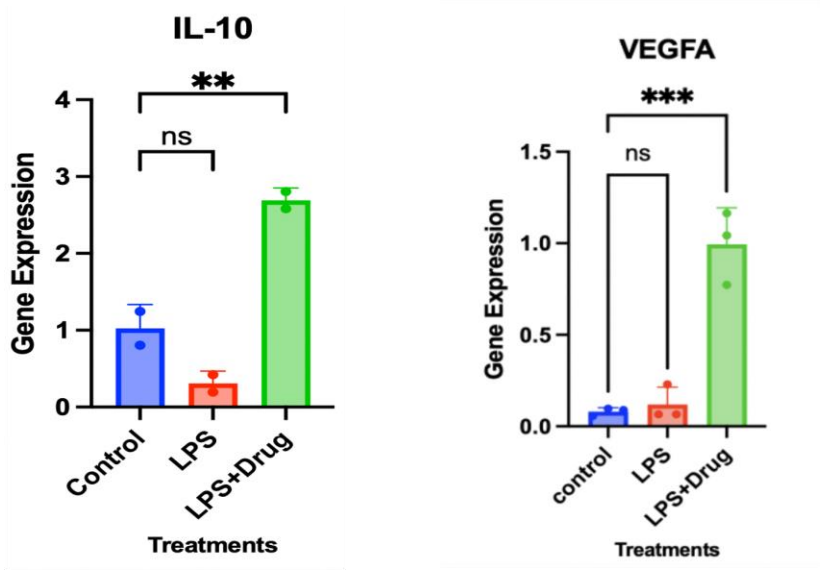


Fig. THP-1 monoculture: IL-10 and MMP7 expression across treatment groups.

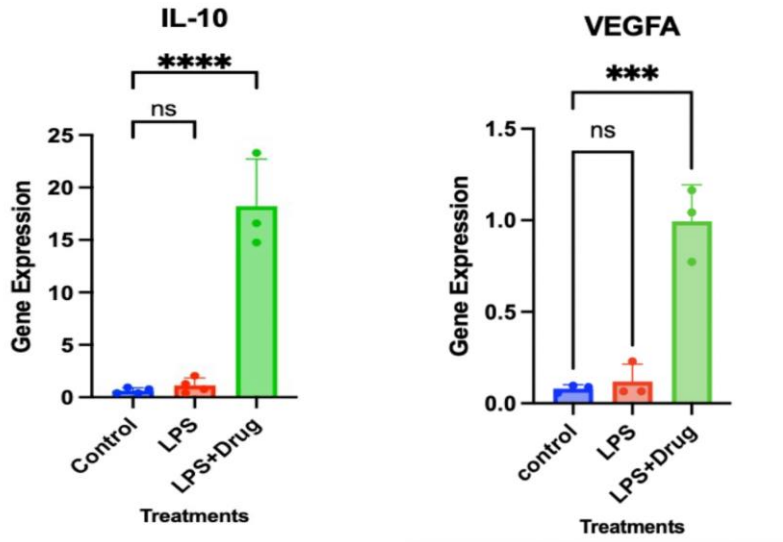


Fig. THP-1–HaCaT co-culture: IL-10 and VEGFA expression across treatment groups.

THP-1 monoculture: LPS alone did not significantly alter IL-10 (ns) but significantly reduced MMP7 (**p<0.001). LPS + nanomedicine significantly increased both IL-10 (*p<0.01) and MMP7 (***p<0.0001).

Co-culture: LPS + nanomedicine significantly increased VEGFA expression relative to control and LPS alone, alongside the IL-10 increase already shown, supporting a pro-angiogenic, repair-associated shift.

MMP7 supports matrix remodeling rather than excess deposition; VEGFA supports the vascularization needed for organized repair — together with IL-10, a consistent pro-healing signature.

Conclusions & Next Steps

KEY FINDING

Nanomedicine treatment significantly increased IL-10 across models, increased MMP7 in THP-1 monoculture, and increased VEGFA in THP-1–HaCaT co-culture- supporting M2 macrophage polarization and a matrix-remodeling, pro-angiogenic response, and validating the skin-on-a-chip platform as a viable testbed.

NEXT STEPS

1. Expand the gene panel	Add M1 markers (TNF-α, IL-6) and a fibrosis marker (COL1A1) for a fuller expression profile
2. Evaluate functional healing	Add cell migration assays to assess functional wound-closure behavior, not just gene expression
3. Integrate 3D bioprinting	Move from a flat co-culture to a bioprinted 3D dermal layer for greater physiological realism
4. Increase replicates & timepoints	Add timepoints and biological replicates to strengthen statistical power

Acknowledgements

Thank you to my PI and mentor for their guidance throughout this project:

Zahra Sabet — PI

Dr. Kerui Wu — Mentor

Joint School of Nanoscience and Nanoengineering (JSNN)

FUNDING ACKNOWLEDGEMENT

This work was supported by the National Science Foundation REU Award #2349519.

References

Sources cited for background statistics and macrophage biology on slides 2–3.

1. Amici, J.M., Taïeb, C., LeFloc'h, C., Demessant-Flavigny, A.-L., Seité, S., & Cogrel, O. (2022). Prevalence of scars: an international epidemiological survey in adults. Journal of the European Academy of Dermatology and Venereology, 36(10), e799–e800. https://doi.org/10.1111/jdv.18277

2. Ploeger, D.T.A., Hosper, N.A., Schipper, M., Koerts, J.A., de Rond, S., & Bank, R.A. (2013). Cell plasticity in wound healing: paracrine factors of M1/M2 polarized macrophages influence the phenotypical state of dermal fibroblasts. Cell Communication and Signaling, 11, 29. https://doi.org/10.1186/1478-811X-11-29

3. Ferrante, C.J., & Leibovich, S.J. (2012). Regulation of macrophage polarization and wound healing. Advances in Wound Care, 1(1), 10–16. https://doi.org/10.1089/wound.2011.0307

All project-specific content (hypothesis, platform design, methods, and IL-10 data) reflects original work by Jessica Wei and Zahra Sabet.