A pathology-driven *in vitro* model of endothelial dysfunction revealing GEF-modulated oxidative and cytokine dysregulation

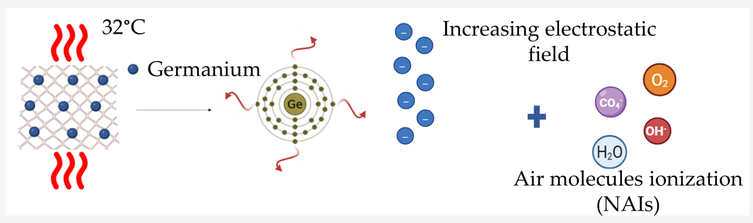
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INTRODUCTION

Chronic inflammation and endothelial dysfunction are central pathophysiological determinants of various chronic diseases, including cardiovascular and joint disorders. **Germanium-Embedded Fabrics** (**GEF**) represent an innovative, non-pharmacological therapeutic platform. These bioactive textiles emit mid- and far-infrared (**MIR/FIR**) radiation and generate negative air ions (**NAIs**) upon thermal activation at skin temperature (~32°C).

Despite emerging clinical evidence supporting GEF in post-surgical recovery and sports injuries, the underlying cellular mechanisms remain poorly characterised. Specifically, how GEF-induced physical stimuli modulate the cross-talk between endothelial and immune cells within complex microenvironments is still unexplored. Conventional 2D cultures fail to recapitulate the spatial and mechanical complexity of vascular tissues, necessitating more physiologically relevant investigative models.



MATERIAL AND METHODS

3D Bioprinting & Perfusion Culture

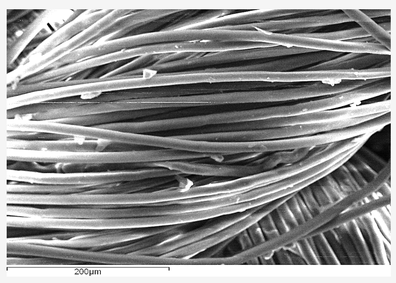
An inflammation and endothelial dysfunction model was fabricated using a Rokit INVIVO 4D2D bioprinter. Human umbilical vein endothelial cells (**HUVECs**) and monocytic **THP-1** cells (1:2 ratio) were encapsulated in 8% **ColMA hydrogel** (0.25% LAP) at 1.5x10^6 cells/mL.

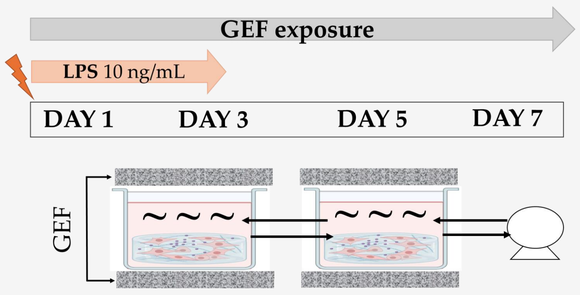
To induce endothelial dysfunction, constructs were pre-treated with LPS (10 ng/mL) for 3 days to trigger monocytic TNF-a release. Scaffolds were maintained for **7 days** in a **perfusion bioreactor** (flow: 1 mL/min) under GEF exposure.

Biological Assays

Cell viability was assessed via **Live/Dead staining** (Calcein AM/PI). Intracellular ROS (superoxide) levels were quantified through **DHE assay** and analysis (DHE/DAPI ratio). **Endothelial activation** was evaluated by Western Blot for **ICAM-1** protein expression (normalized to GAPDH). Total Nitric Oxide (NO) was measured in supernatants via **Griess reaction**. Pro-inflammatory and anti-inflammatory **cytokine profiles** were determined using the Luminex™ 200 **multiplex platform**.



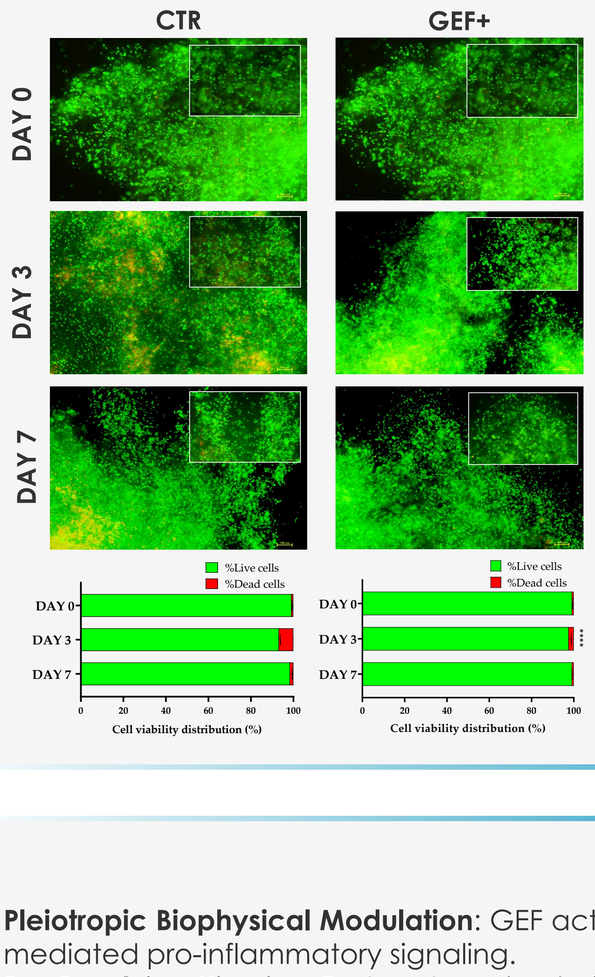




RESULTS

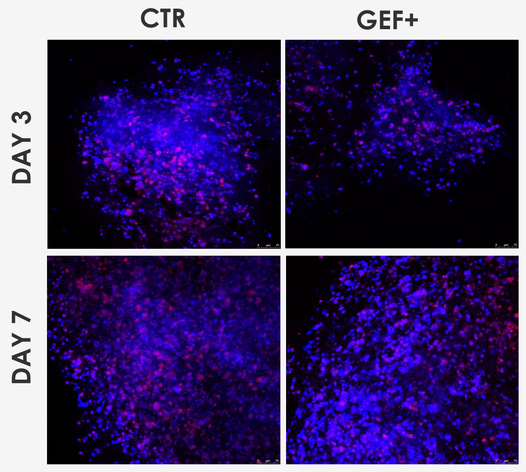
Cell viability (L/D assay)

The bioprinting process using ColMA-LAP bioink maintained exceptional cell viability (99.1% post-printing). At day 3, LPS-induced stress reduced viability to 93.3% in controls, while GEF-treated constructs maintained significantly higher levels (97.6%, $p < 0.001$). By day 7, both groups recovered to >98%, confirming the non-lethal nature of the inflammatory stimulus and the protective role of GEF.



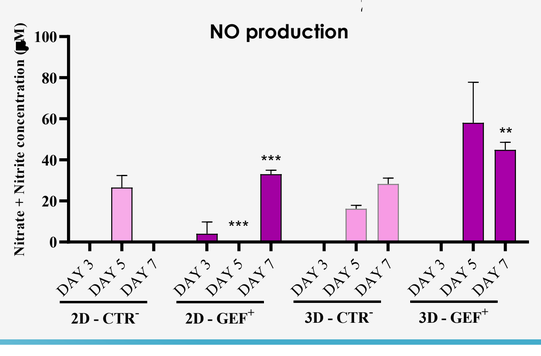
Attenuation of Oxidative Stress

GEF-treated 3D hydrogels exhibited a robust antioxidant phenotype, evidenced by a significant reduction in the DHE/DAPI fluorescence ratio at day 7.



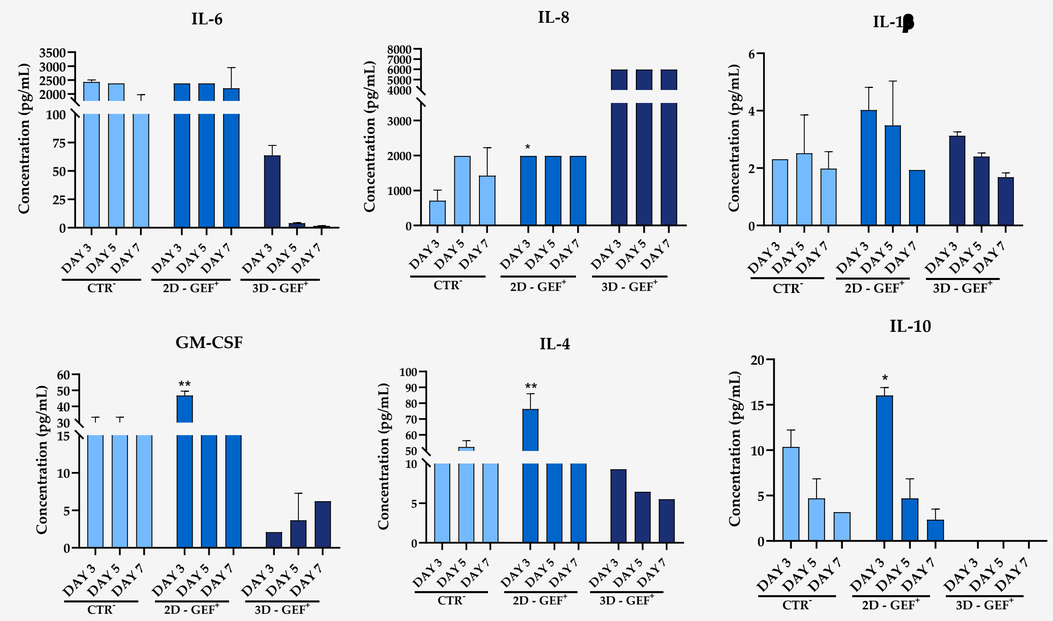
Nitric Oxide (NO) Production

The 3D perfused environment markedly increased NO secretion vs 2D. GEF, peaking at day 7 ($p < 0.01$), indicating stimulated eNOS activity within the biomimetic environment.



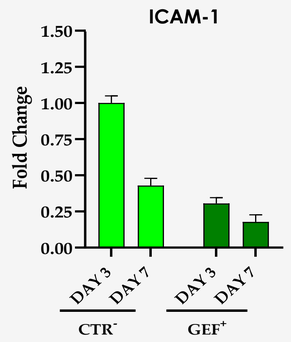
Multiplex Cytokine Profiling

The 3D architecture profoundly reshaped the secretome, showing lower IL-6 and undetectable IL-10 compared to 2D. GEF treatment in 3D suppressed pro-inflammatory mediators (IL-6, GM-CSF) at concentrations orders of magnitude lower than in equivalent 2D cultures, confirming the superior sensitivity of the 3D platform.



Endothelial activation

Western blot analysis confirmed a reduction in ICAM-1 protein levels in GEF-treated 3D cultures at both day 3 and day 7, with the most pronounced attenuation during the peak inflammatory phase (day 3). Notably, this anti-inflammatory trend was properly detectable only in the 3D environment, remaining elusive in 2D monolayer setups.



CONCLUSIONS

Pleiotropic Biophysical Modulation: GEF acts as an integrated modulator, combining NAIs, FIR, and magnetic fields to stabilize the redox balance and suppress NF-κB-mediated pro-inflammatory signaling.

Pro-Resolving Kinetics: Rather than simple immunosuppression, GEF facilitates a biphasic response, accelerating the temporal transition from endothelial activation (ICAM-1 upregulation) to inflammatory resolution.

Vascular Homeostasis: The significant boost in Nitric Oxide (NO) levels in 3D constructs suggests that GEF effectively stimulates eNOS activity under physiologically relevant flow conditions.

3D Model Superiority: The bioprinted 3D platform demonstrated higher biomimetic fidelity and sensitivity than 2D systems, revealing subtle molecular trends and cytokine dynamics closer to in vivo conditions.Clinical Implications: These findings provide a robust mechanistic foundation for using GEF-based wearables as an adjunctive strategy for managing chronic vascular inflammation and tissue repair.

REFERENCES

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For further informations: