A 3D Printed Stamp for In-vitro modeling

Objective

Chronic wounds affect over 6.5 million people in the US, with healing hindered by hypoxia. Current treatments like Hyperbaric Oxygen Therapy (HBOT) are limited by the lack of scalable, physiologically relevant in vitro models[1]. Existing systems often isolate stressors, lack reproducibility, and are resource-intensive.

We developed an in vitro assay that simulates chronic wounds by combining a low-nutrient environment with a 3D-printed stamp to create uniform abrasions in 96-well plates seeded with human keratinocytes[2]. Success is defined by <10% scratch variation and a significant wound closure difference in the control and treatment groups. This system improves reproducibility, reduces resource use, and supports quantitative analysis of hypoxia-driven healing[3].

Concept design and engineering constraints

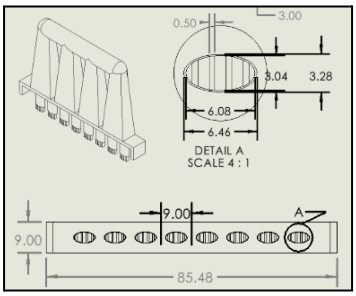
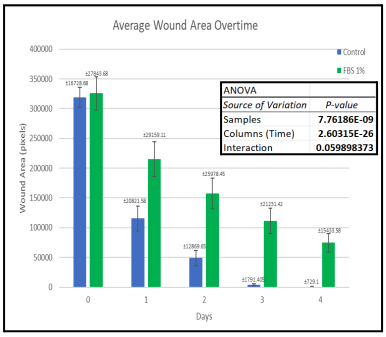


Figure 1: Prototype design of the stamp

To improve chronic wound modeling, we developed a 3D-printed PLA stamp with four elliptical extrusions and plate guides that create uniform scratches across eight wells of a 96-well plate. This design maximizes cell engagement while minimizing cell use, enabling scalable, high-throughput modeling. Unlike inconsistent pipette tip methods, the stamp ensures reproducible mechanical injury. A current limitation is the use of HaCaT cells, which lack full native wound-site fidelity.

Prototype Development



Methods: HaCaT cells were seeded (120,000/well) in a 96-well plate. After confluence, a 3D-printed stamp created four uniform scratches per well. Scratches were verified and imaged via fluorescence microscopy. Wells were treated with 10% (control) or 1% FBS (low-nutrient environment), and closure was tracked every 24 hours. ImageJ and MATLAB quantified wound area and closure.
Results: Control wounds closed significantly faster. Two-way ANOVA showed media type and time were significant ($p < 0.05$), with no interaction ($p > 0.05$). Minimal error bars confirmed reproducibility

Conclusion

We developed a stamping device that creates four uniform scratches per well in 96-well plates, enabling consistent mechanical injury in keratinocyte monolayers. Future work aims to improve durability, standardize use, and integrate oxygen assays under hypoxic, nutrient-deprived conditions.

[1] Kang HJ et al. J Control Release. 2021;333:176–187.
[2] Seo MD et al. Biomol Ther (Seoul). 2012;20(2):171–6.
[3] Kosol W et al. Biochem Biophys Res Commun. 2020;522(2):335–341.