3D Cell Assemblies to Evaluate Inflammatory Response to Dual-agent Delivery Platforms Targeting Bacterial Vaginosis

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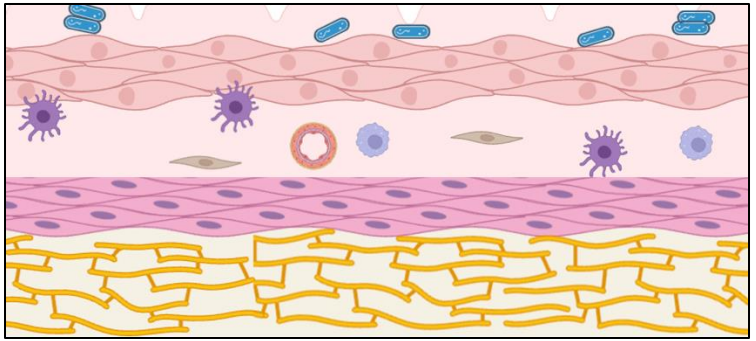
NEXT-GENERATION DELIVERY INNOVATIONS

Background: Epidemiology

- 29% of women are affected by Bacterial Vaginosis (BV) globally with a recurrence rate of 76%

- Dysbiosis of the lower female reproductive tract (FRT), where the predominant commensal bacteria(*Lactobacillus spp.*) is replaced with diverse pathogenic bacteria



- BV leads to a more basic vaginal pH (>4.5), disruption of the epithelial barrier, cellular inflammatory response, and an increase of immune cells to the FRT

- *Gardnerella* is present in 95% of BV cases

Background: Immune Response

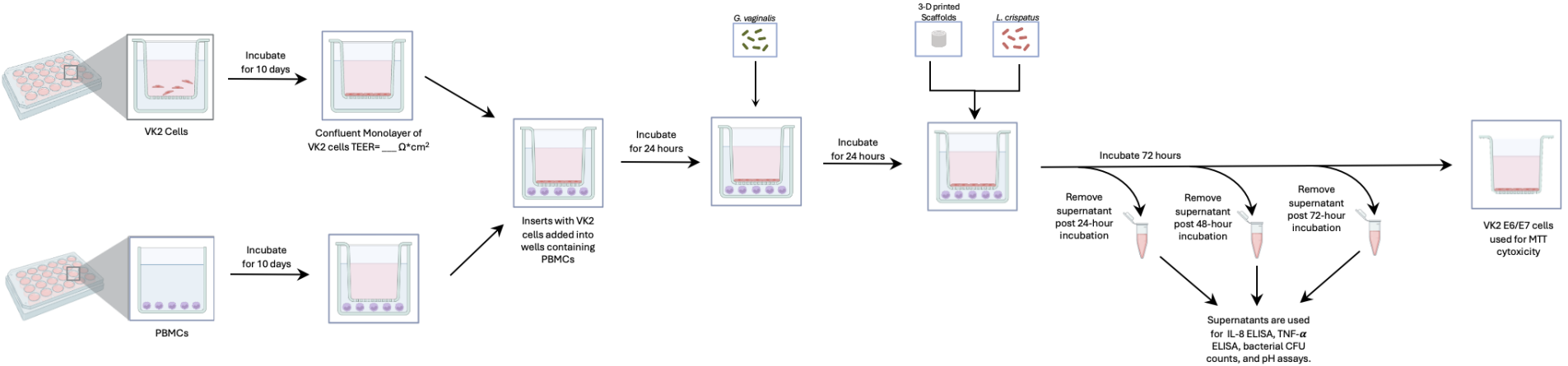
- *Gardnerella* elicits a pro-inflammatory response in vaginal epithelium
- Produces short fatty acid chains, which enhances toll-like receptors and increases cytokine production in host cells
- Acetate and Succinate (other BV species) inhibit immune cell chemotaxis
- pH >5 causes higher production of cytolytic enzymes
  - -Vaginolysin
  - -Sialidase
  - -Prolidase
- Immune response increases susceptibility to adverse health effects, including higher susceptibility to STDs and pregnancy complications

Goals of this study

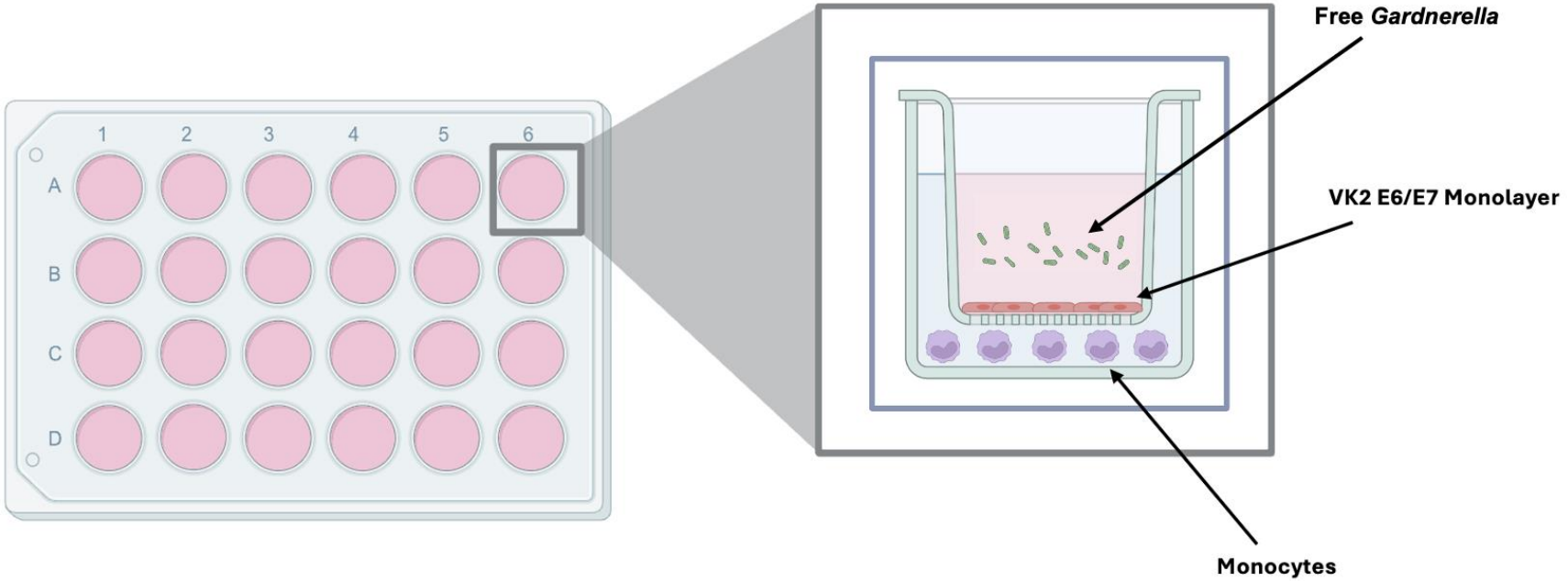
- Platforms for local delivery of both antibiotic and probiotic have recently been proposed to promote user adherence and avoid recurrence.

- This study designed a novel 3D cell assembly to assess lower FRT cell inflammatory response to *Gardnerella* and to platforms delivering combined therapies, such as dual-agent antibiotics (metronidazole) and probiotics (*L. crispatus*).

Methods

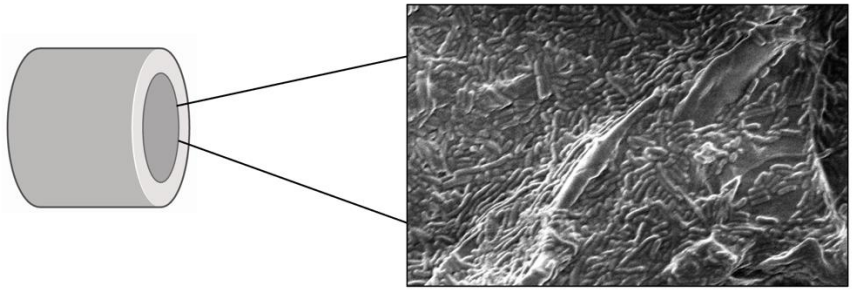

VK2 Cells
Incubate for 10 days
Confluent Monolayer of VK2 cells TEER= ___ Ω*cm²
PBMCs
Incubate for 10 days
Inserts with VK2 cells added into wells containing PBMCs
G. vaginalis
Incubate for 24 hours
3-D printed Scaffolds
L. crispatus
Incubate for 24 hours
Incubate 72 hours
Remove supernatant post 24-hour incubation
Remove supernatant post 48-hour incubation
Remove supernatant post 72-hour incubation
VK2 E6/E7 cells used for MTT cytoxicity
Supernatants are used for IL-8 ELISA, TNF-α ELISA, bacterial CFU counts, and pH assays.

Dual Co-Culture Model

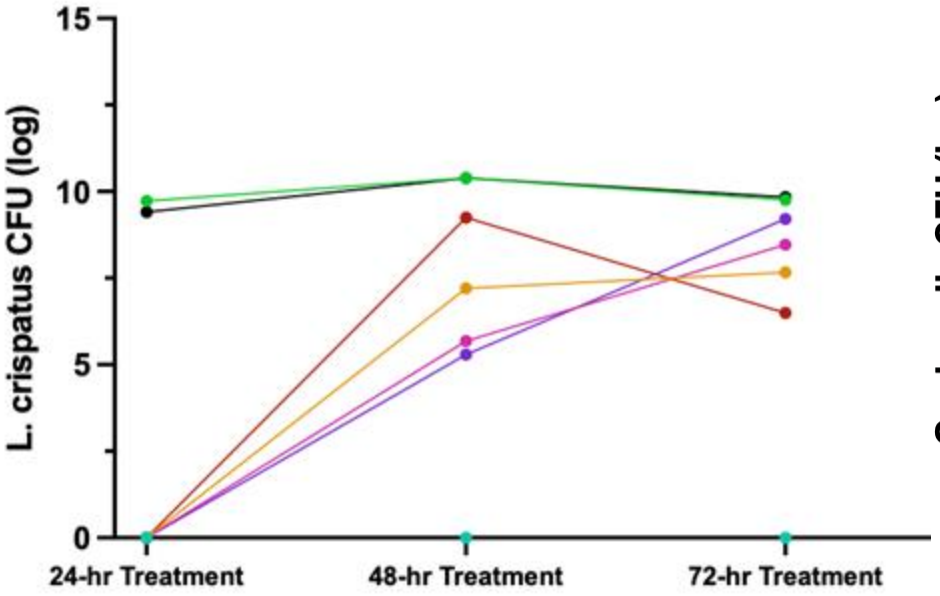


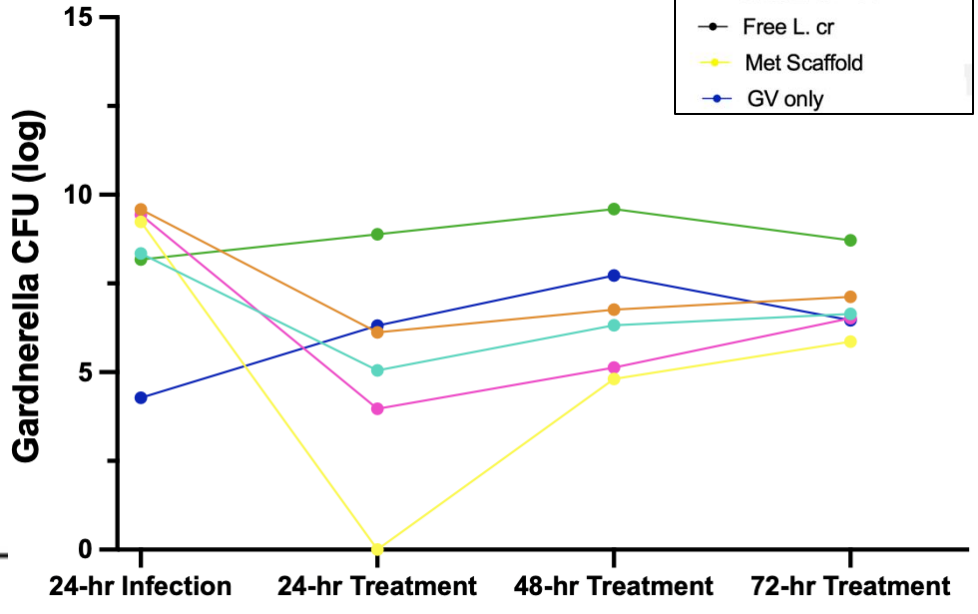
Dual Delivery Scaffolds

- Non-adherence and antibiotic resistance require further treatment
- Silicone 3-D printed Scaffolds containing metronidazole shell and *Lactobacillus crispatus* core
- *Gardnerella* knockdown seen in previous literature after treatment of dual *L. crispatus*-metronidazole scaffolds



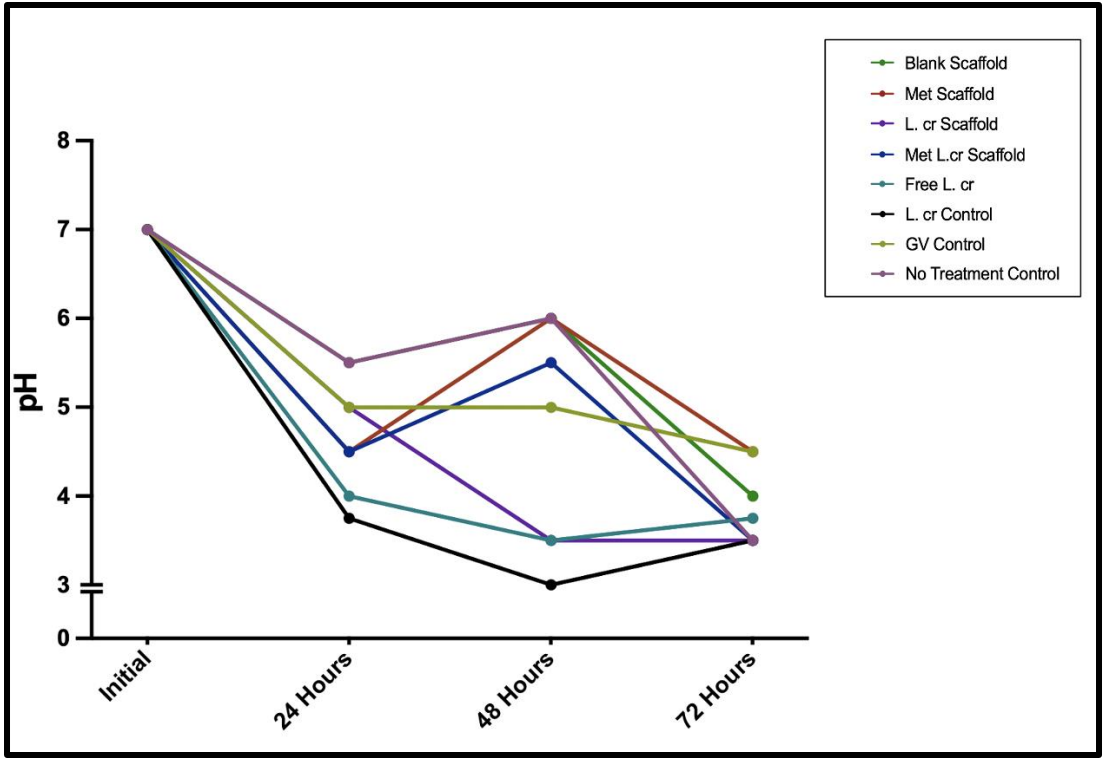
Results: *Lactobacillus crispatus* vs. *Gardnerella*



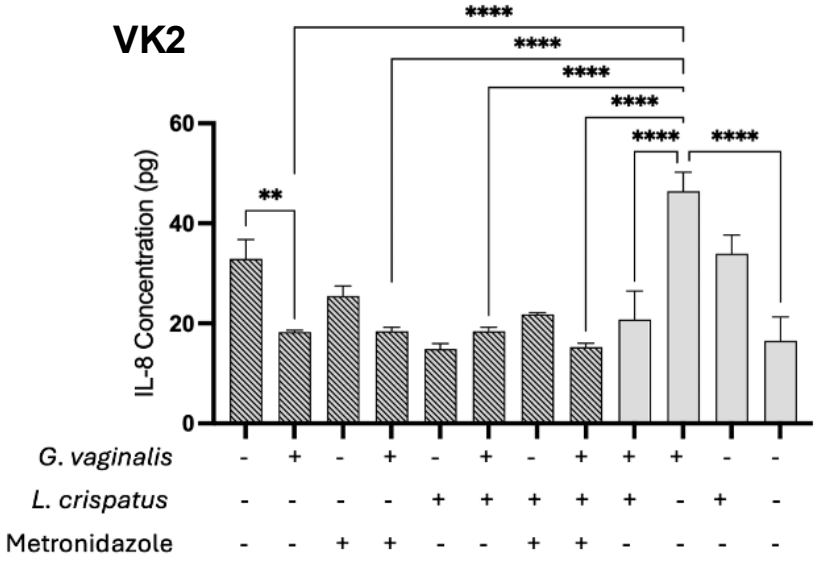


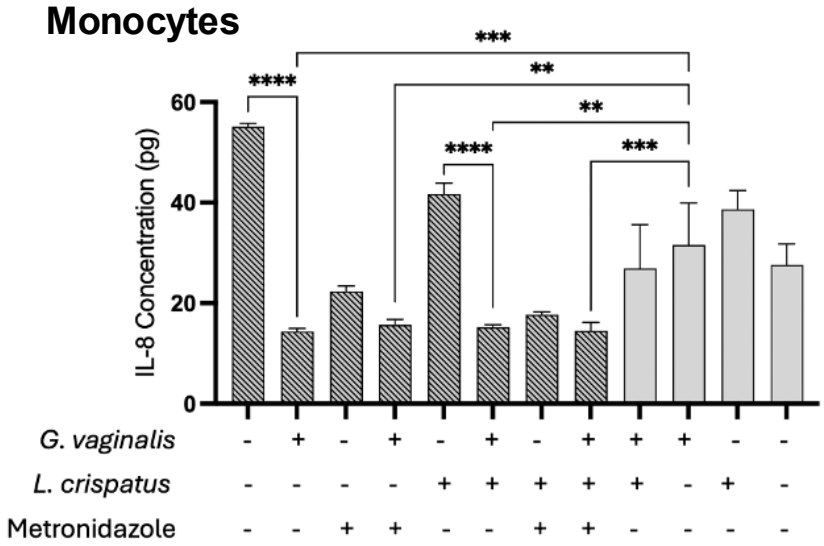
Results: pH Change

- KSFM media neutral pH of 7
- *Gardnerella* control remains above 4.5
- *L. cr* control drops below 4 and remains below 4
- *L. cr* scaffold lowest pH for treatment groups
- Met Scaffolds large increase then drop at 48-hr

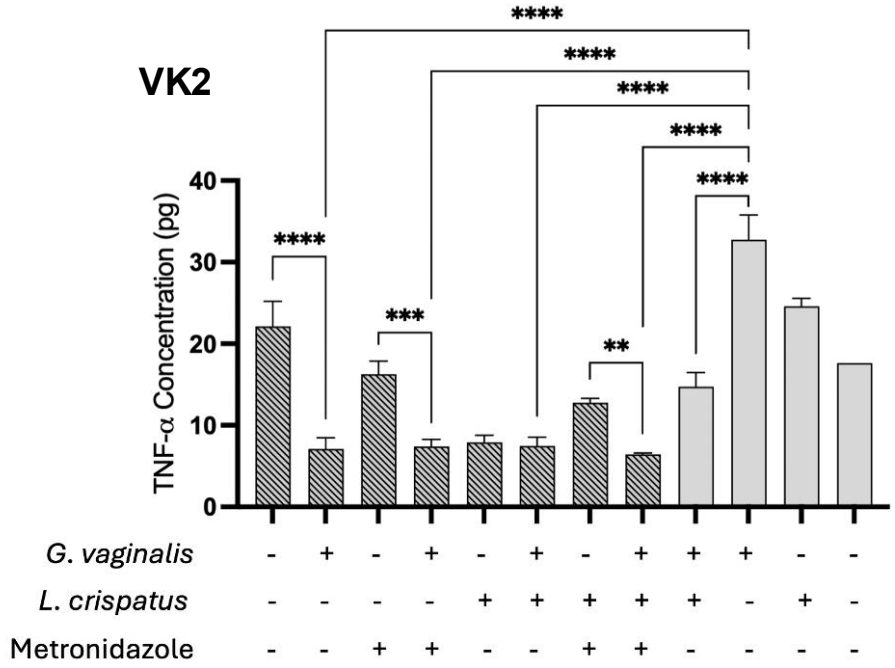


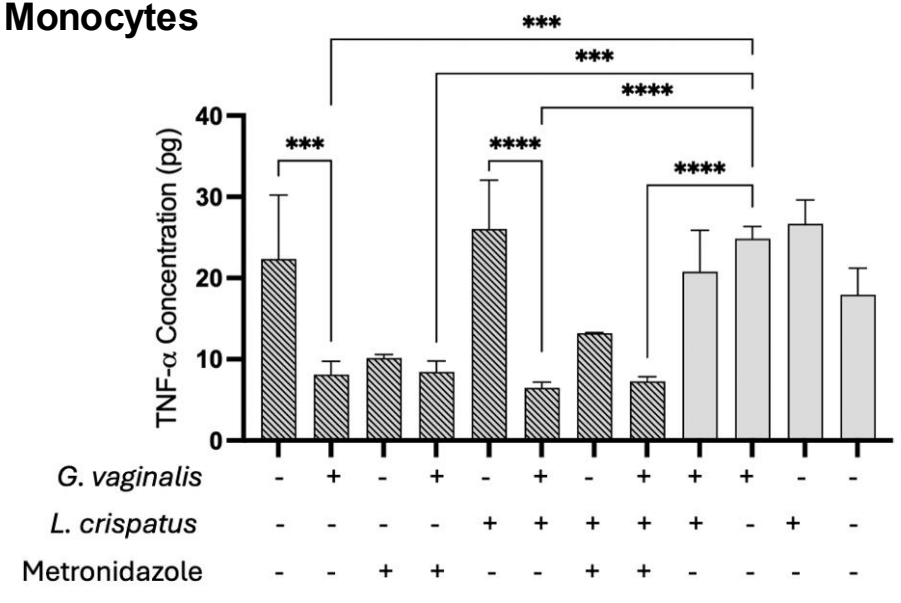
Results: Il-8 Release Post 72-Hour Treatment





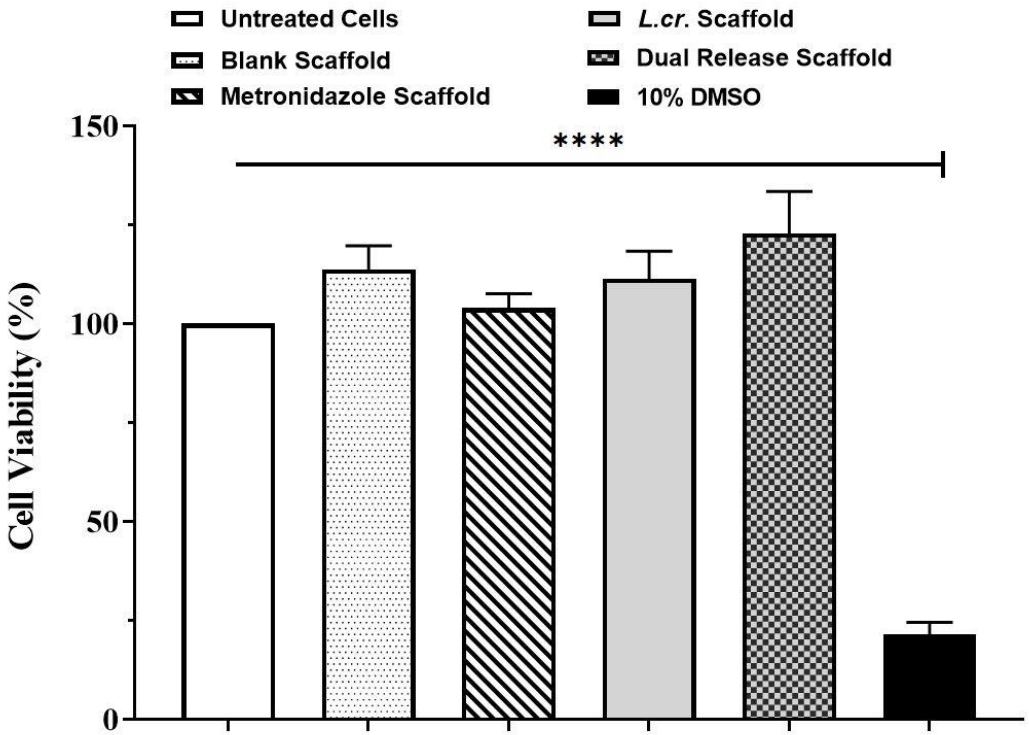
Results: TNF-α Release Post 72-Hour Treatment





Results:
VK2 E6/E7 Viability

- MTT analysis after treatment
- No cytotoxicity of VK2 cells after treatments with scaffolds, *L. crispatus*, or metronidazole



Summary and Future Work

- *Gardnerella* inhibits Il-8 and TNF-α for the first 48 hours.
- After 72 hours *Gardnerella* elicits a large increase in cytokine release
- Metronidazole scaffolds induce a complete knockdown in *Gardnerella* CFU
- Dual agent scaffolds elicit the least cytokine release
- Overall, after 72 hours all treatments prevent inflammatory response
- This work establishes the viability of a novel 3D cell assembly modeling the FRT to examine in a controlled environment the effects of dual-agent probiotic and antibiotic delivery methods to locally treat BV.

Acknowledgements

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