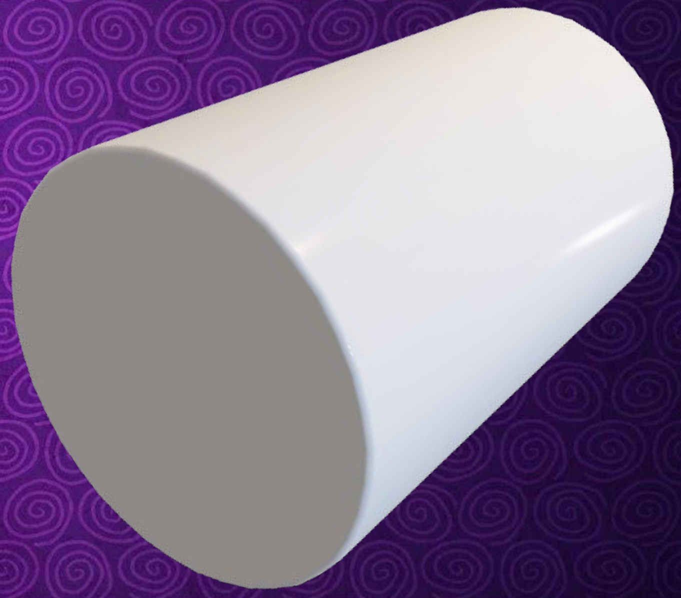
Development and Characterization of 3D-Printed Silicone Scaffolds for Antibiotic Release to Treat Bacterial Vaginosis

Anthony Kyser[1], and Jill M. Steinbach-Rankins[1, 2]

[1]Department of Bioengineering, J.B. Speed School of Engineering;
[2]Center for Predictive Medicine University of Louisville;

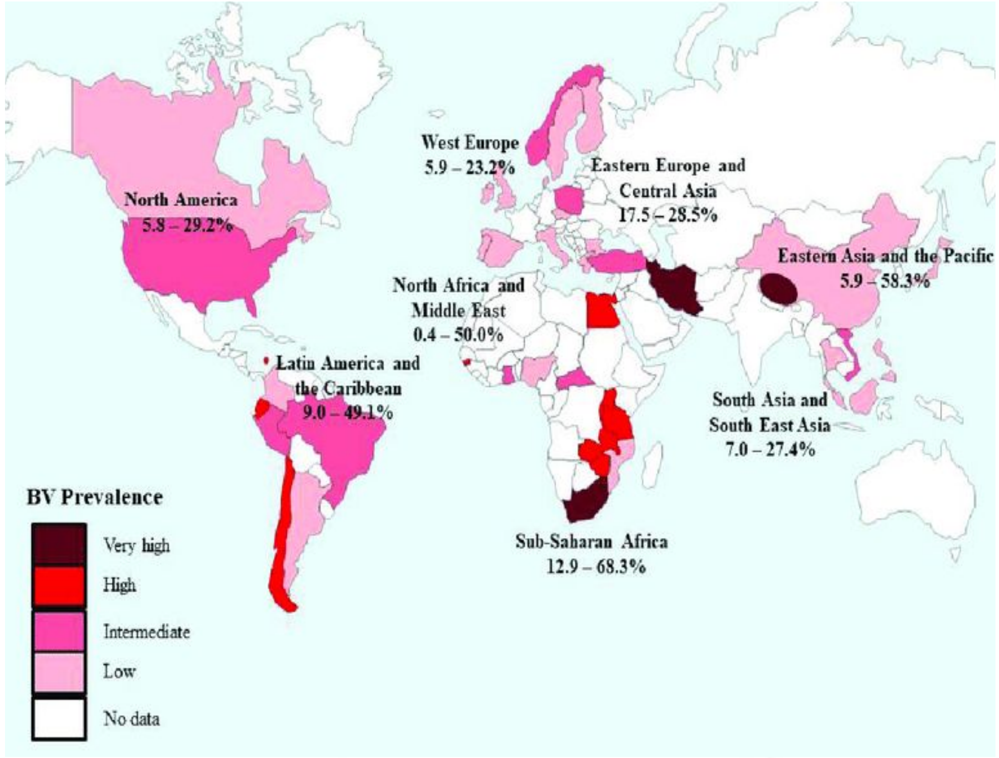


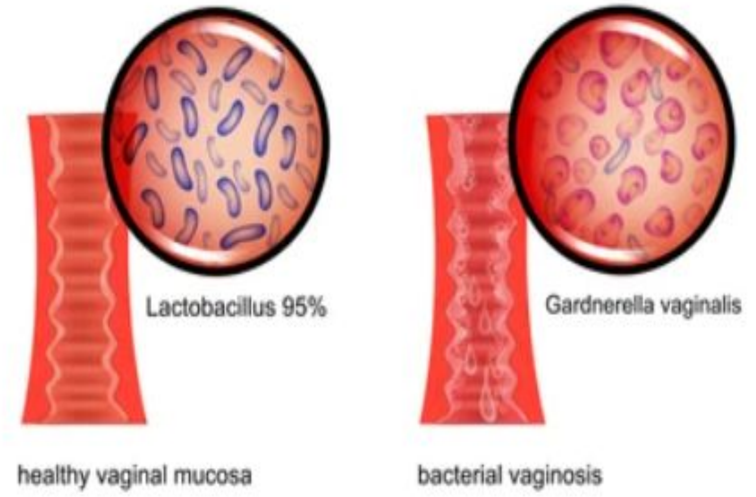
Background: Epidemiology

- 29% women of reproductive age in North America are affected by bacterial vaginosis and 25% of women globally
- Bacterial vaginosis is caused by imbalance between pathogenic, symbiotic, and commensal bacteria resulting
  - Higher pH
  - Shift in diversity and proportion of anaerobic bacteria
- Healthy lactobacillus-dominant microbiota
- Prominent organisms include *Gardnerella vaginalis (G.v.)* and *Prevotella bivia* and an absence of *Lactobacillus*
- Compromises vaginal immune response and increase susceptibility to viral and bacterial sexually transmitted infections
- Cost of treatment: North America: $3.6 billion USD;
  Global: $4.8 billion USD; half of costs are from recurrent cases

https://styledme.com/purple-zoom-background/
https://www.shutterstock.com/image-vector/vector-illustration-vagina-affected-by-bacterial-556874716
https://www.researchgate.net/figure/Global-burden-of-bacterial-vaginosis-2013-Data-extracted-from-a-systematic-review_fig2_330035220/download

Prevalence of BV, 2013





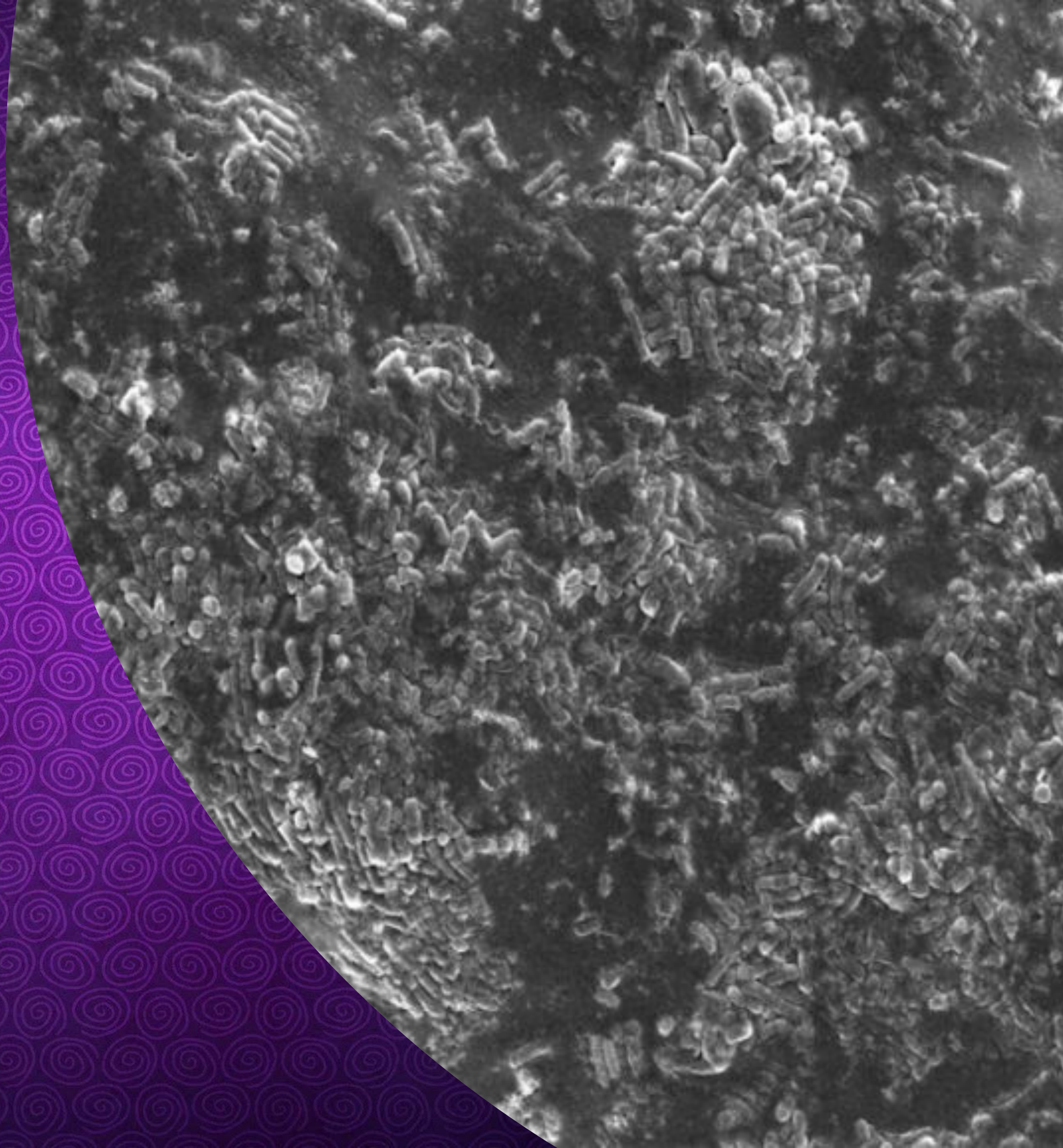
Background:
Current Treatments

- Antibiotics: metronizadole, tinidazole, and clindamycin
- Oral or vaginal application for 5-7 days
- Eliminate healthy bacteria and foster antibiotic-resistance
- Antibiotic resistance and lack of clinical adherence contributes to the 66% recurrence rate one-year post-infection
- Topicals have been shown to be more effective with 63% bioavailability; however, topicals need to be applied frequently
- Need for new agents that maintain and restore vaginal health

https://www.theguardian.com/society/2017/oct/13/antibiotic-resistance-could-spell-end-of-modern-medicine-says-chief-medic

Background: Probiotics

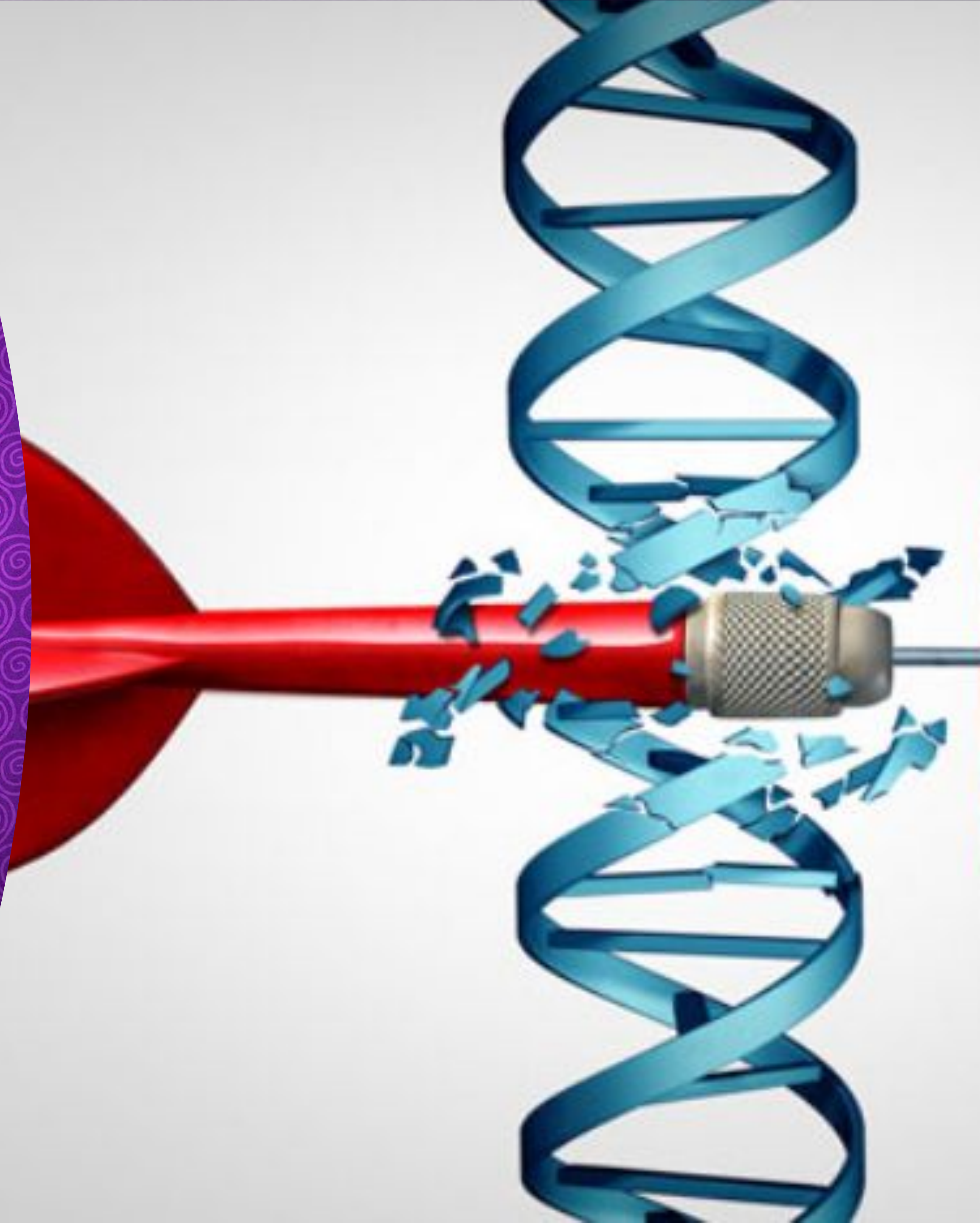
- *In vitro*, *in vivo*, and clinical studies have shown promise for probiotics to prevent/treat BV infection and restore vaginal health
- *Lactobacillus acidophilus, Lactobacillus crispatus,* and *Lactobacillus reuteri* produce lactic acid, providing a healthy acidic microenvironment
- Additionally, complementary therapies such as topical metronidazole followed by oral probiotics have shown 85% effectiveness against BV compared to 45% with antibiotic alone
- However, reproducible results in larger and well-defined trials must be considered before recommendation as therapy



https://www.uptodate.com/contents/bacterial-vaginosis-treatment

Background: Solutions through Drug Delivery

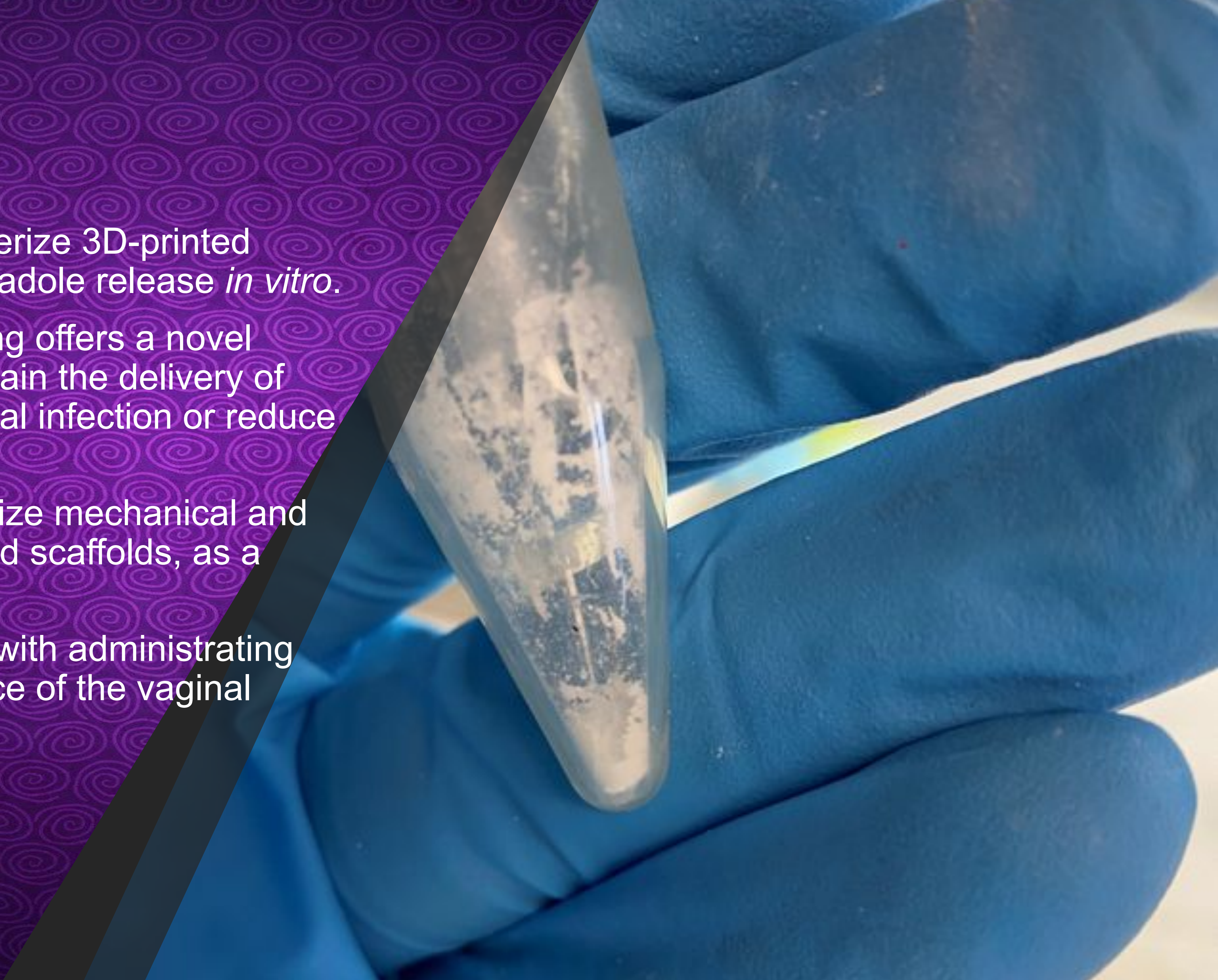
- Few studies have focused on the development of 3D-printed platforms for sustained release (here, antibiotics) to the FRT
- Current delivery platforms consist of vaginal creams/gels
- Few long-term delivery options exist that enable less frequent application, sustained therapeutic effect, and potentially improved user adherence
- 3D-printing can be utilized to fabricate scaffolds for sustained delivery in a high-throughput method
- A variety of inks promote scaffold stability, sustained release, and co-delivery of multiple agents
- 3D-printing enables different architectures and personalization for co-delivery strategies



https://www.europeanpharmaceuticalreview.com/article/77469/faster-development-delivery-systems/

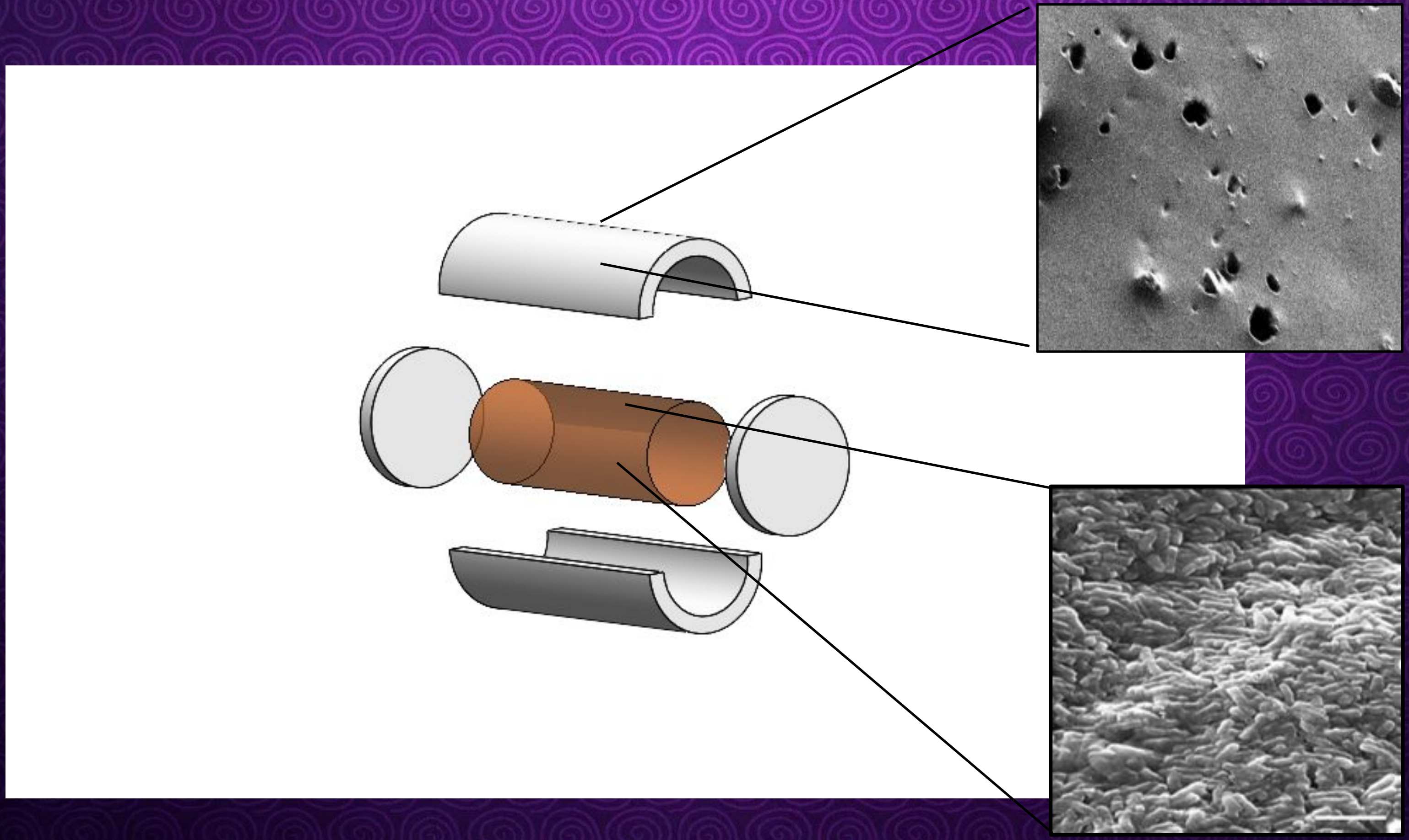
Study Goal

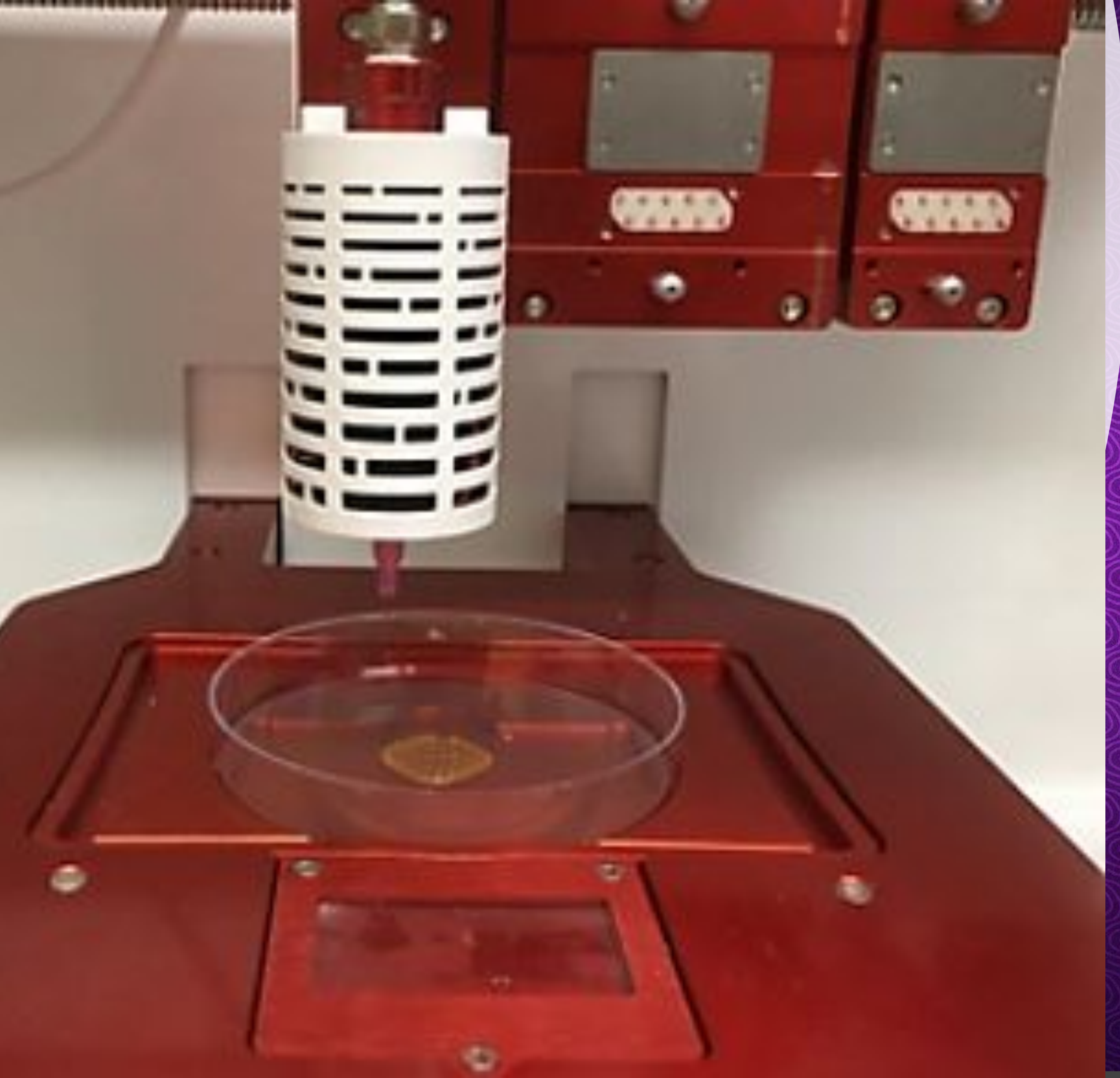
- Goal: To fabricate and characterize 3D-printed scaffolds that sustain metronizadole release *in vitro*.
- We hypothesize that 3D-printing offers a novel approach to intravaginally sustain the delivery of antibiotics, to prevent/treat initial infection or reduce recurrent BV infections.
- Short-term goal is to characterize mechanical and release properties of 3D-printed scaffolds, as a function of curing conditions.
- Long-term goal is to follow up with administrating probiotics to restore the balance of the vaginal microbiota.



Dual-Delivery of Antibiotic and Probiotic

- Through fabrication of a 3D-printed scaffold with sustained release, we propose a dual drug delivery system composed of an antibiotic shell and probiotic core
- SEM images provide promising evidence for release of probiotic through antibiotic shell





Methods

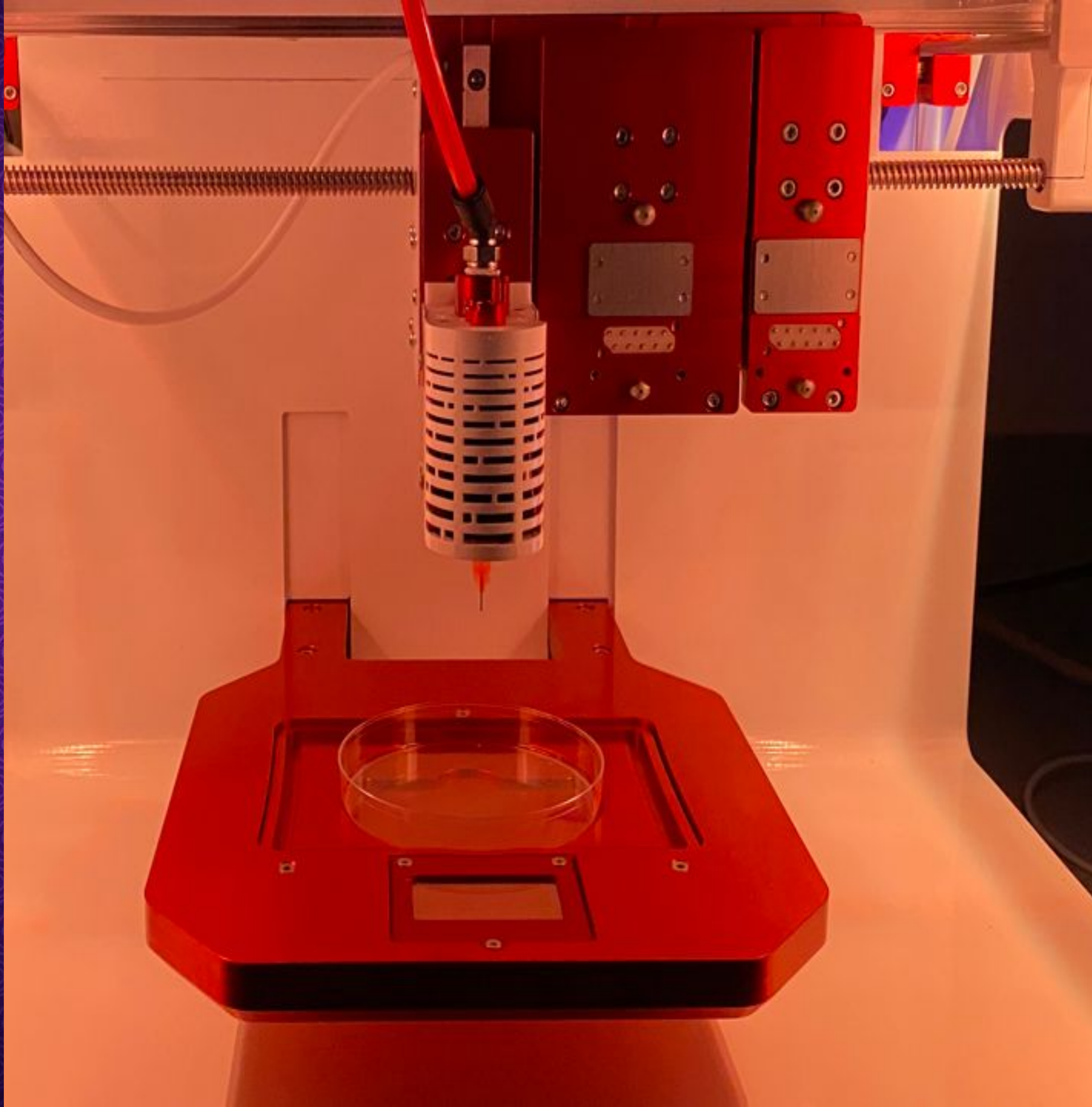
Multiple facets of 3D-Printed scaffold:

- Formulation of silicone ink
- Optimization of printing and curing parameters
- Printing resolution
- Sustained metronizadole release
- Mass loss and swelling
- Mechanical structures
- Cytotoxicity to vaginal epithelial cells

Formulation

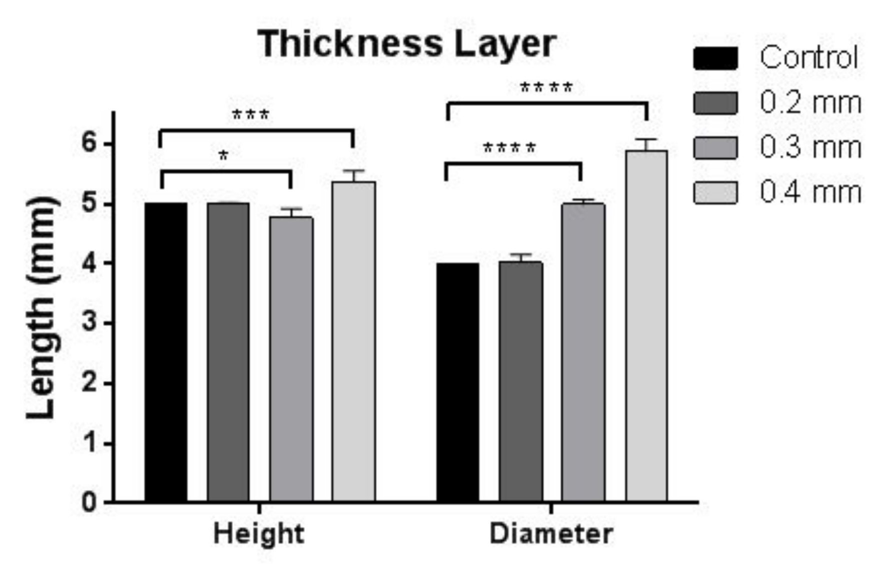
- Vinyl-terminated polydimethylsiloxane (70%) and vinyl, methyl-modified silica (30%)
- Trimethylsiloxane-terminated methylhydrosiloxane-dimethylsiloxane
- 50 μg/mg metronizadole
- Dimethyl sulfoxide (DMSO)

- Scaffolds cured under various conditions including:
    - 24 hr at 50°C
    - 4 hr at 50°C + 24 hr at 20°C
    - 4 hr at 50°C + 72 hr at 4°C



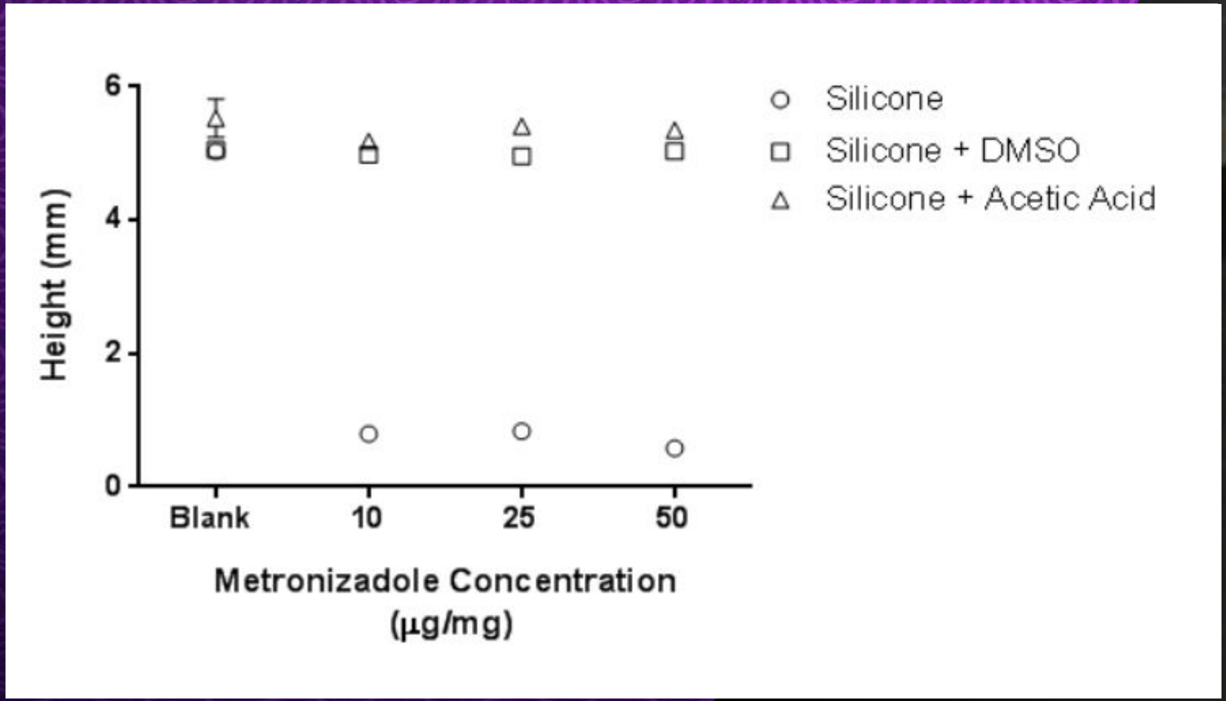
Results: Silicone Print Resolution

- Initial design conceived in SOLIDWORKS®
- Printed height and diameter were evaluated to determine attainable resolution based on the printing parameters
- Parameter ranges tested:
  - Formulation and Needle size: 16, 23, 26, 30G
  - Pressure: 15-120 psi
  - Temperature: 15-45°C
  - Extrusion Rate: 2.5-7.5 mm/sec
  - Thickness Layer: 0.2-0.4 mm



Results: Print Resolution of Metronizadole Scaffold

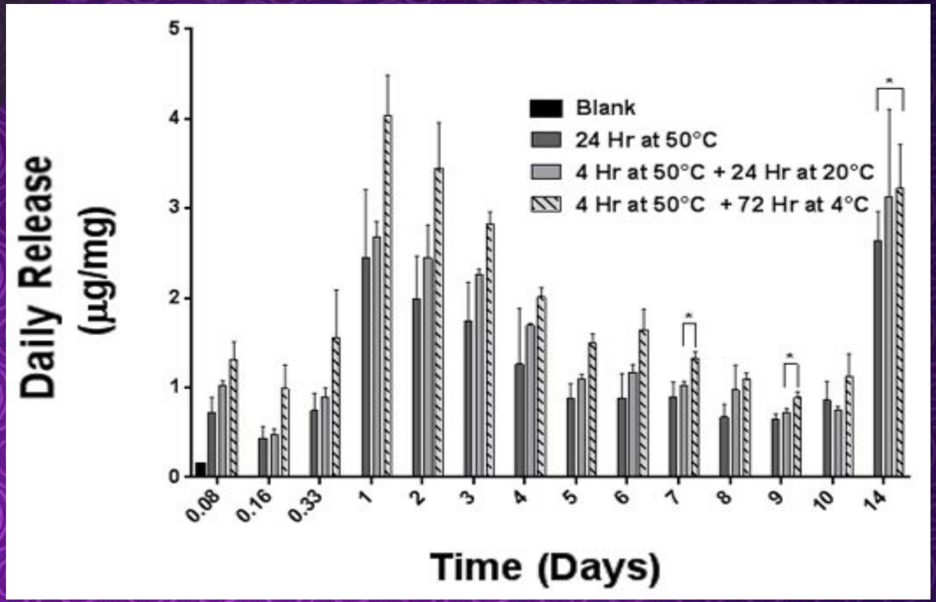
- Utilization of multiple formulations to develop homogenous antibiotic (metronizadole) ink
- Printing diameter and height were evaluated to determine attainable resolution based on the metronizadole loading
  - 10, 25, 50 µg/mg

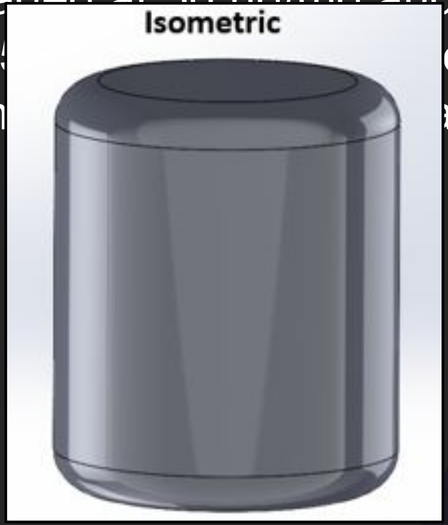


Results: Development and Metronizadole Release

- A cured scaffold is advantageous:
  - Mechanical integrity
  - Enables sustained-release
  - May provide an environment for healthy cell proliferation

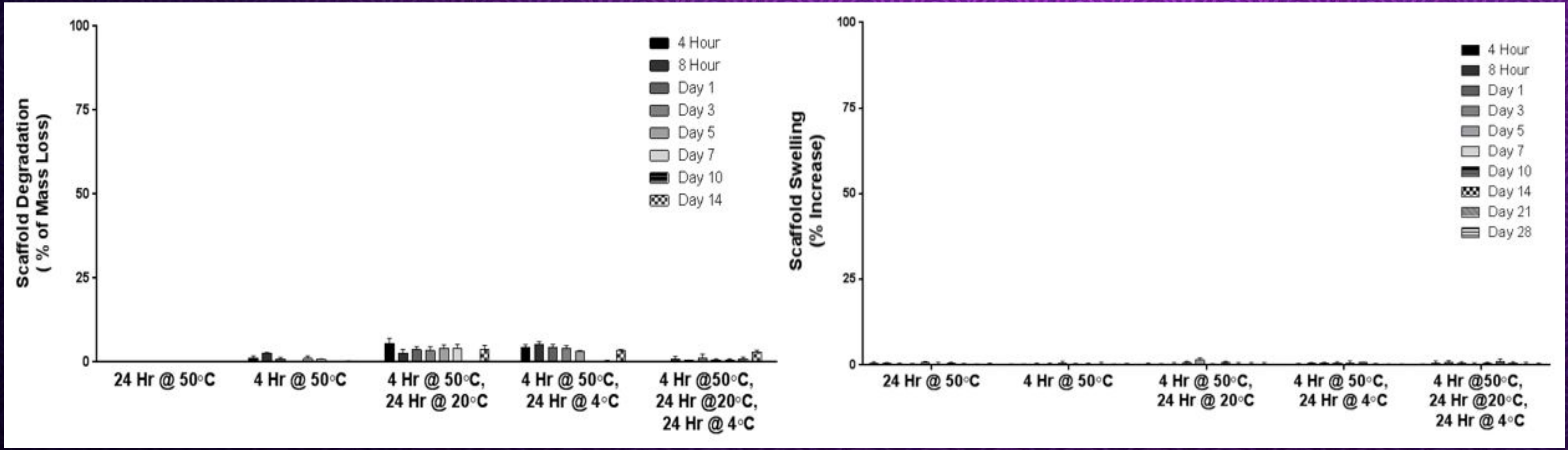
- Scaffolds shown to be most advantageous loaded at 50 µg/mg and cured at 24 hr at 5 … of 4.0 and 27.0 µg/m … ays, respectively





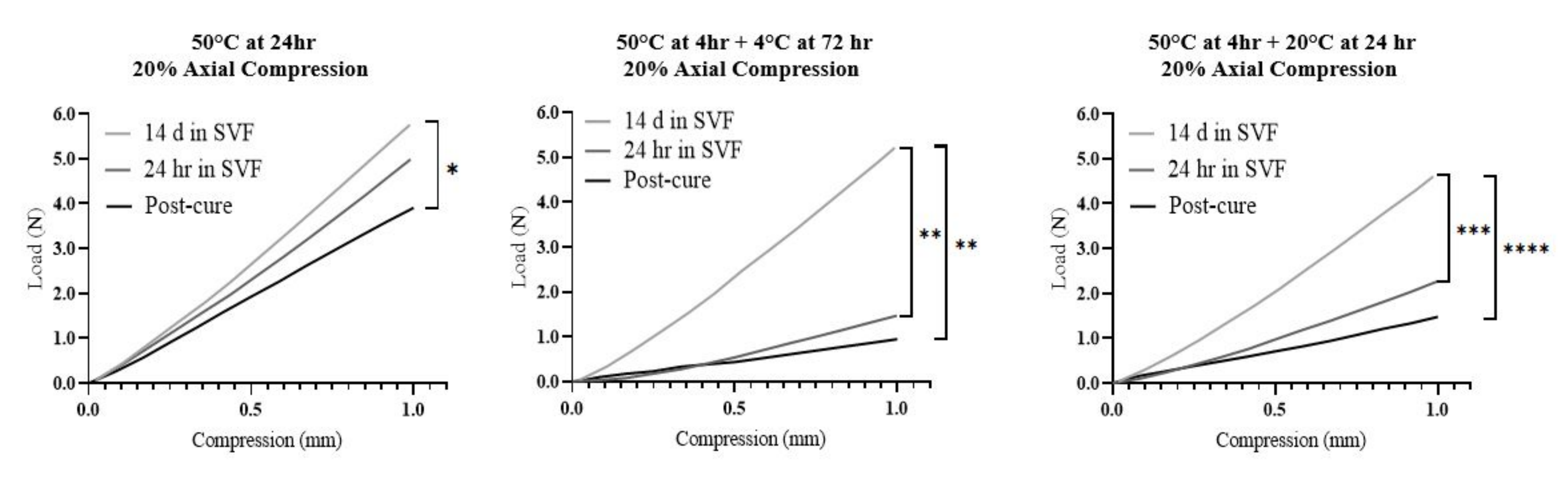
Results: Effect of Curing on Mass Loss and Swelling

- Scaffolds were evaluated for swelling and degradation across different curing parameters corresponding to release studies
- Negligible mass loss (<6%) throughout all curing parameters
- Negligible swelling (<2%) throughout all curing parameters



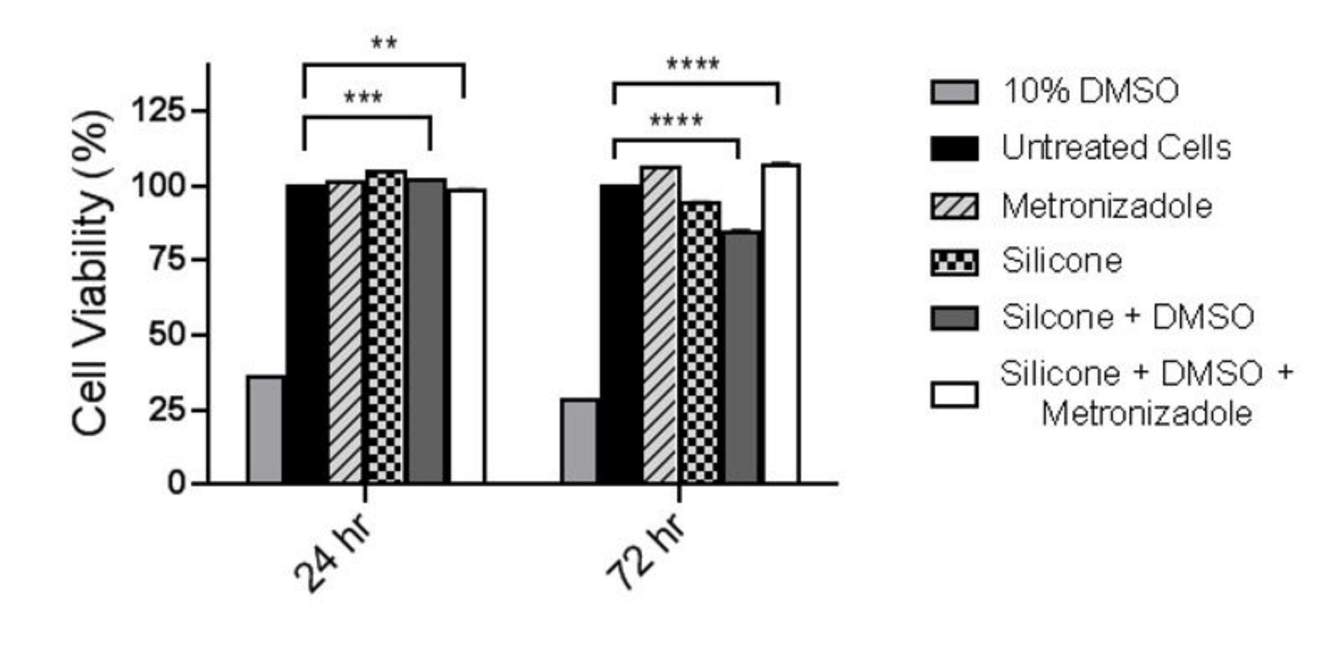
Results: Effects of Curing on Mechanical Integrity

- Scaffolds were evaluated for mechanical integrity across different curing parameters corresponding to release studies
- Scaffolds demonstrated elastic behavior under 20% compression and 4.0 N load



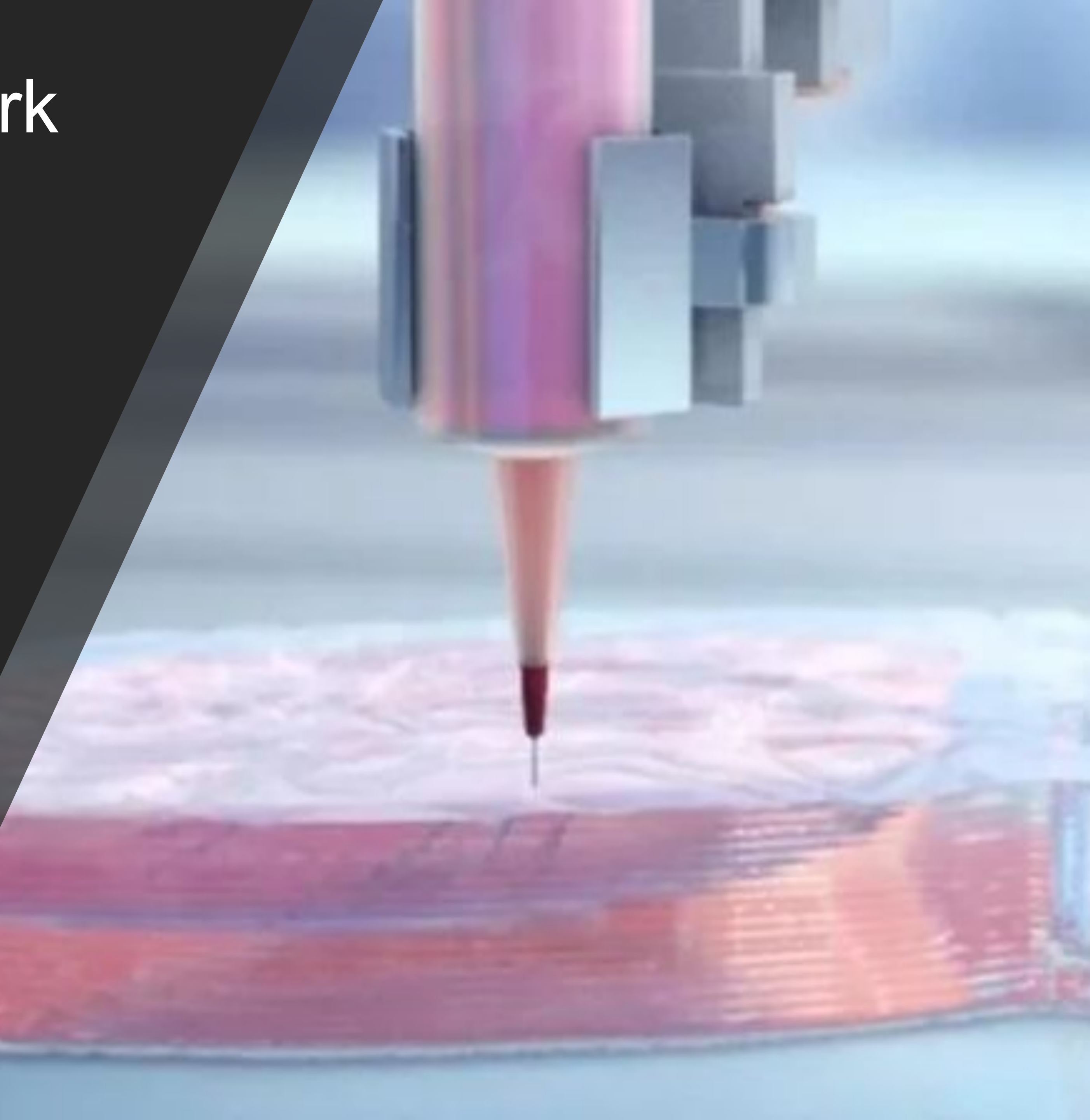
Results: VK2/E6E7 Viability

- Formulated scaffolds were administered for 24 and 72 hr to vaginal keratinocytes (VK2/E6E7)
- An MTT assay was used to determine cell viability
- Negligible cytotoxicity was observed in treated keratinocytes -- comparable to untreated cells
- Future studies will assess the cytotoxicity and cytokine profiles in EpiVaginal tissue



Summary and Future Work

- Early data from this study show that 3D-printed silicone metronizadole scaffolds may be fabricated to provide:
    - fine printing resolution
    - high antibiotic loading
    - sustained release
    - stable mass loss and degradation throughout 14 days
    - initial biocompatibility with vaginal epithelial cells
- Ongoing work is focused on completing mechanical and biological inclusion characterization
- Future work will assess the impact of 3D-printed scaffolds on BV infection *in vitro* and *in vivo* to help treat/restore female reproductive health
- For the first time, we show that 3D-printed metronizadole-containing scaffolds may provide a new alternative to provide sustained antibiotic delivery
- Provides the baseline for a dual-antibiotic probiotic delivery platform



https://all3dp.com/2/what-is-3d-bioprinting-simply-explained/

Acknowledgements & Questions

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