Optimization of cell culture conditions and growth factor dose for the deposition of a bone extracellular matrix layer on Poly(propylene fumarate) biomaterials

OSTEO ENGINEERING LAB
Department of Plastic Surgery | OSU

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

Authors: Ryan S. Sefcik[1], Tyler J. Bishop[1], Ruchi Mishra[1],John P. Fisher[2], David Dean[1]
Institutions: [1]Department of Plastic Surgery, The Ohio State University, Columbus, OH; [2]Fischell Department of Bioengineering, University of Maryland, College Park, MD

Introduction

Background:

Light-based 3D printed poly(propylene fumarate) (PPF) scaffolds have been developed for bone regeneration. These scaffolds have smooth, somewhat hydrophobic surfaces. It has been previously shown that PPF can be used as a biomaterial with no cytotoxicity issues. In addition, growth factors have previously been used to promote cell proliferation and differentiation.

Hypothesis:

We hypothesize that the addition of growth factors to the cell culture media will promote the proliferation and differentiation of human MSCs. Also, we expect that growth factors will enable the cells to coat the surface of the scaffold and achieve a Extracellular Matrix (ECM) of 30-50 µm more quickly.

Objective:

Determine a method to fabricate thin film PPF polymer scaffolds that will not show any cytotoxicity to cells. Then, growth factors will be used to promote cell proliferation and differentiation to create an Extracellular Matrix (ECM) thickness of 30-50 µm.

Materials and methods:

Live-dead assay

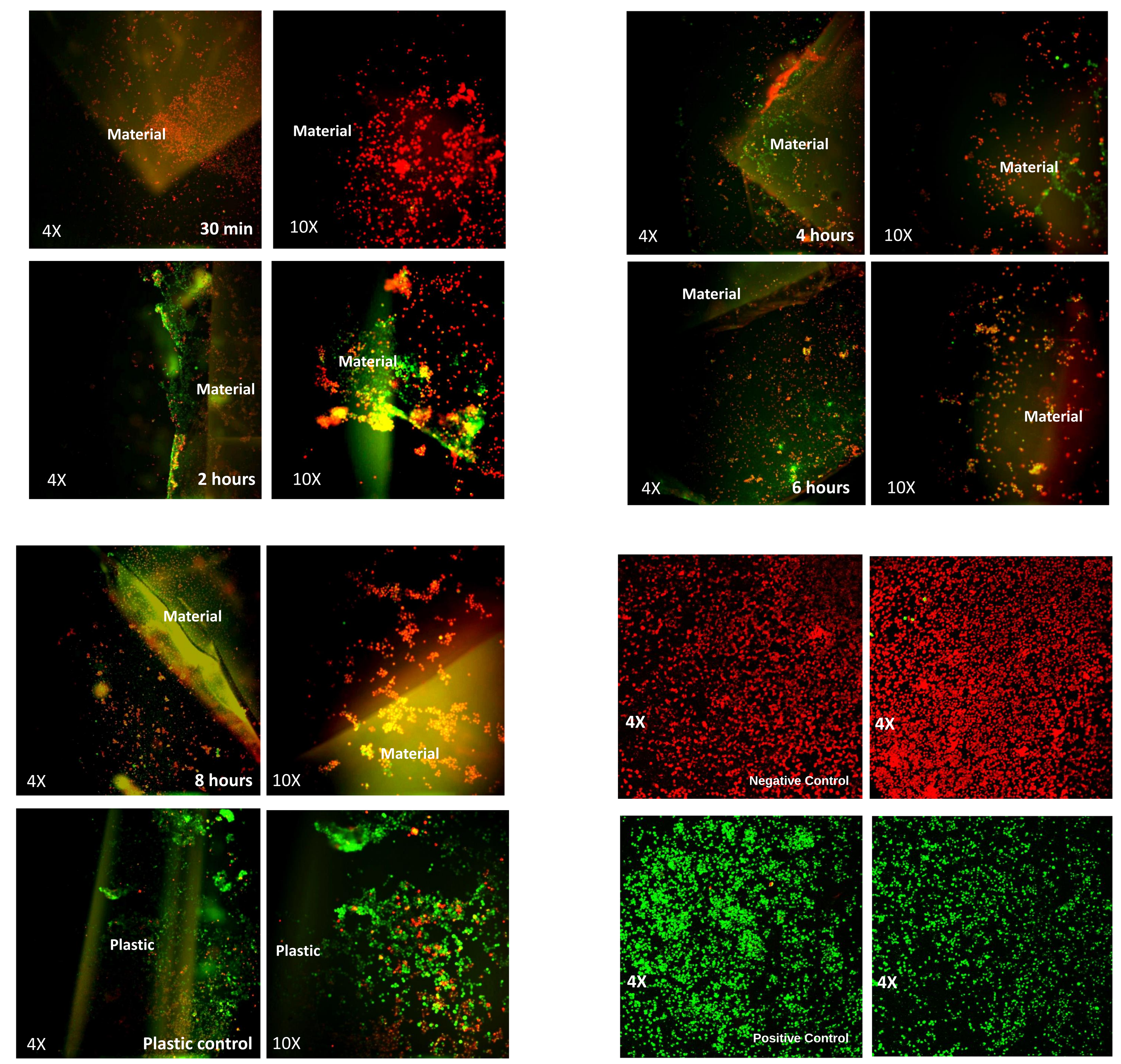
- PPF coupons of 1 x 1 x 0.01 cm^3 dimensions, each composed of 1200 Da PPF, 3% Irgacure 819 (BAPO), 0.7% Oxybenzone (HMB), and 0.4% Irgacure 784 were cured in a UV light box for a set amount of hours.
- L929 fibroblast cells were seeded onto tissue culture plastic well plates.
- After each well was confluent, a PPF coupon was place on top of the cells and it was left for 24 hours.
- Live-Dead staining for direct contact assay was performed to determine if cytotoxicity is present in the coupons.
- Initially, the sample groups were 30 min and 2 hour but it was expanded to 4, 6, and 8 hours to explore the role of cure time on cytotoxicity.
- Then, an additional wash was attempted on the 8 hour coupons to explore the role of washing on cytotoxicity.

Toluidine Blue staining for cell seeding density

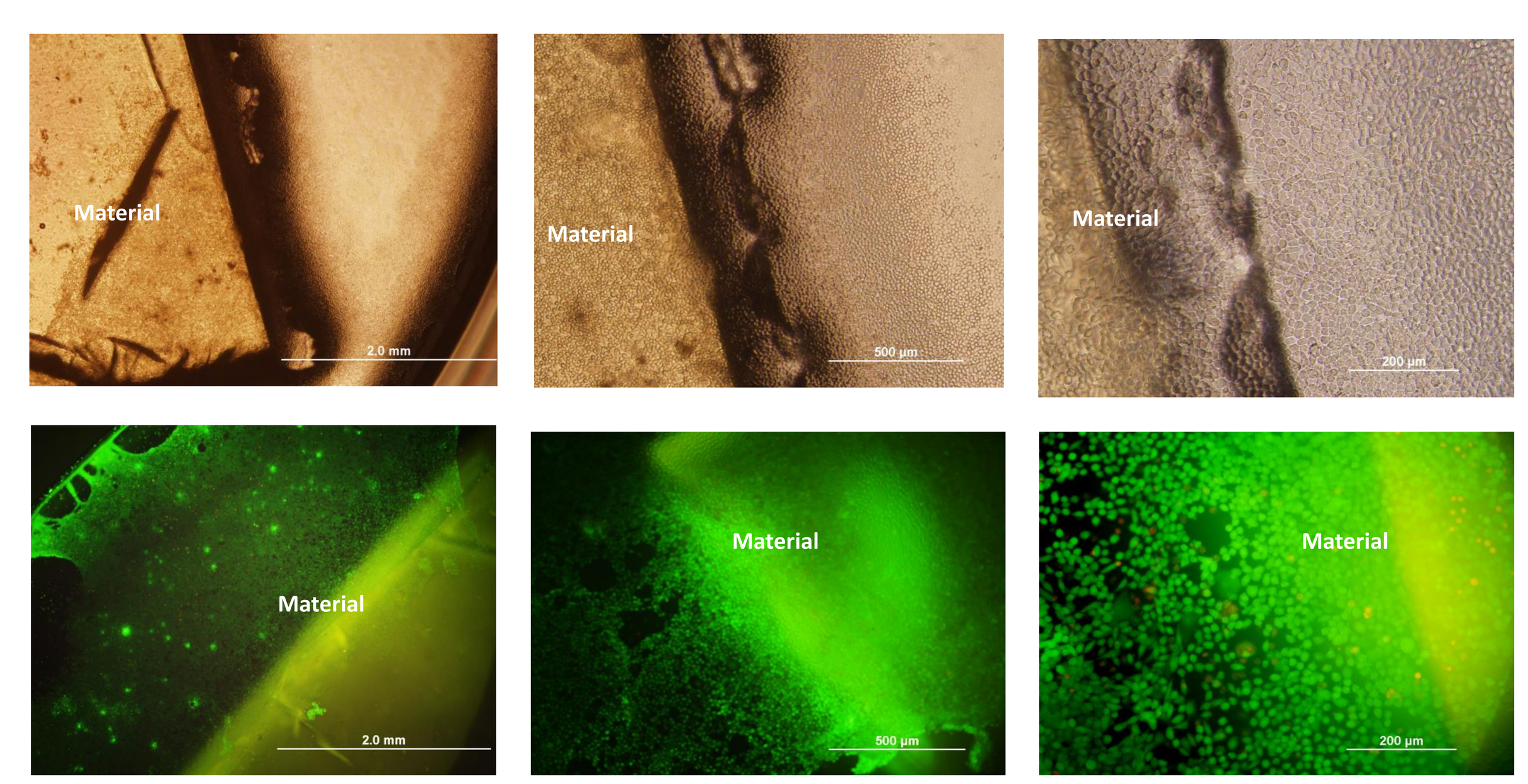
- Canine MSCs were used to better mimic Human MSCs behavior.
- Cells were seeded onto coupons at densities of 10,000, 25,000, 35,000, and 50,000 cells/coupon
- After 4 hours, the cells were fixed with 3.7% Paraformaldehyde and stained using 1% Toluidine Blue.
- Samples were viewed using both macroscopic and microscopic techniques

Results

Live-dead assay for 70% acetone wash + sterilization

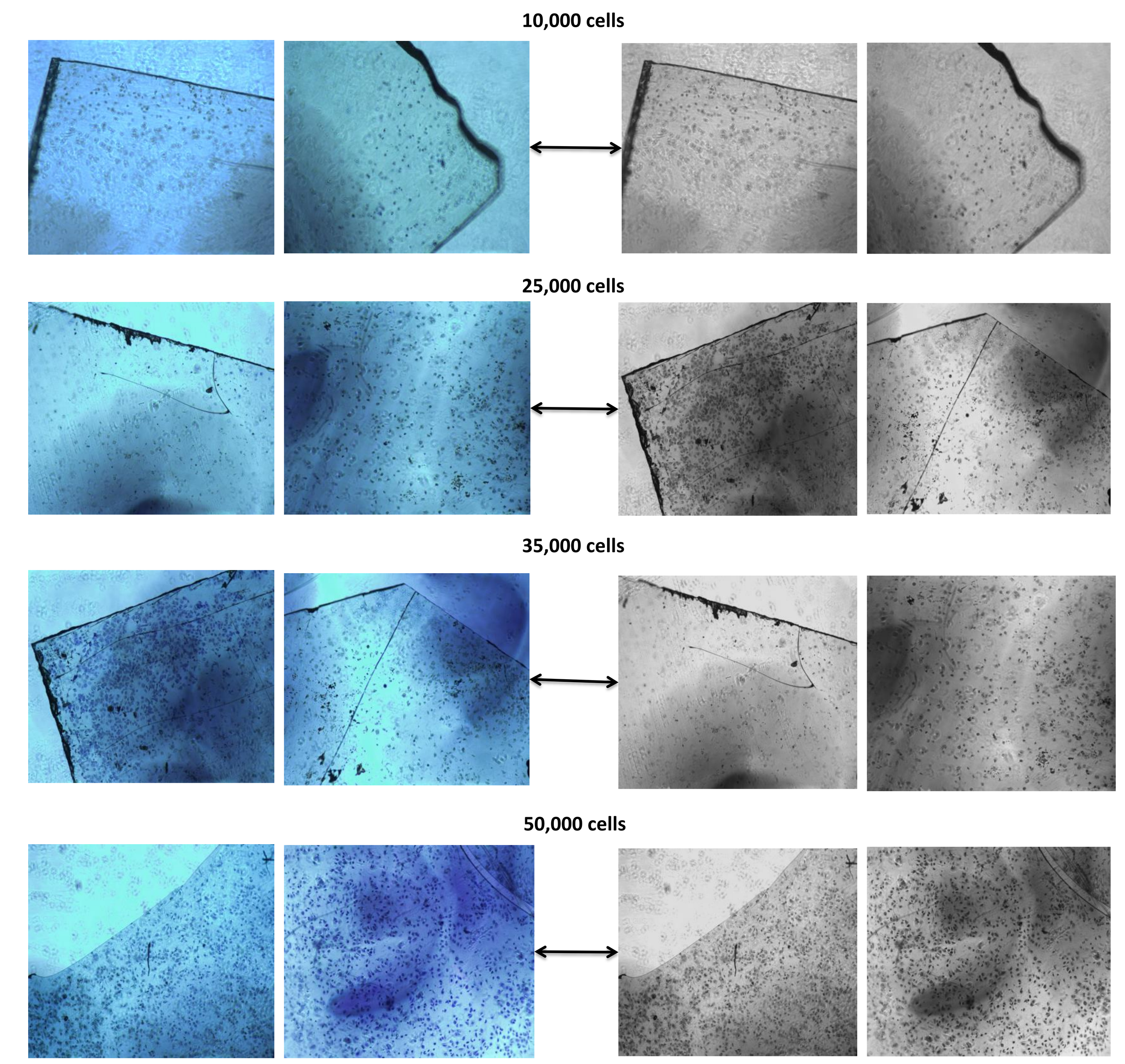


Live-dead assay for 70% acetone wash + sterilization + 70% acetone wash (8hr cure time)



Note: "Double-washing" appears to have rid the samples of cytotoxicity

Toluidine Blue staining to determine cell seeding density



Note: All images presented at 4X magnification

Conclusions

- Polymer cytotoxicity is significantly decreased by incorporating a second wash after gas sterilization.
- Curing time plays a very small role in cytotoxicity.
- A higher cell seeding density will allow the cells to coat the surface more quickly.
- A cell seeding density of 25,000 cells will be appropriate for the study.

References

Acknowledgments