3D-Printed Poly (lactic acid) Scaffolds for Regenerative Medicine: Does PLA Degrade, Support Osteoblastic Cellular Growth?

B. Henninger, S. Seman, R. Rotello, T.L. Norman
School of Engineering and Computer Science, Cedarville University, Cedarville, OH

Introduction
A major challenge facing researchers in the field of regenerative medicine is the ability to repair tissue defects and stimulate healing. Today, bone is often replaced with biologically inert materials such as titanium. Often the more desirable tissue engineering approach would be to provide a scaffold seeded with the osteogenic capability for cell proliferation and growth eventually leading to tissue restoration. The overall goal of our research is to develop a method using three-dimensional (3D) printing to create biodegradable scaffolds of customizable stiffness which promote bone (osteoblasts) ingrowth. In a previous investigation, candidate scaffold design were created using host friendly poly (lactic acid) (PLA) that provided near “trabecular bone like” stiffness required to stimulate cell growth and bone healing (Fig. 1) (Cole et al., 2018). Cole et al. (2018) found that all architectures allowed fibroblast cells (a highly prolific connective tissue cell) to attach and proliferate. Using

Objectives
The X scaffold (Fig. 2) were selected for this project due to customizable stiffness and demonstrated culturability. Utilizing this 3DP PLA scaffold specimen, the objectives of the project were to:
1) assess PLA’s sustainability in a physiological-like environment, i.e. does PLA degrade with soaking time;
2) observe if bone precursor cells (osteoblasts) can attach and survive on PLA scaffolds.

Experimental Methods
Assessment of PLA Sustainability
Eight X scaffold specimens were selected for the assessment of PLA sustainability due to soaking in cell culture media. Specimens were weighed, measured and mechanically tested at the onset of the protocol (t = 0 weeks) and at weeks 1-7 and week 10 while continuously soaking in cell culture media in well plates (Fig. 3). Three measurements were made for each specimen including the width, depth and height.

Results and comparisons are presented here for week 0 and week 10 where the magnitude of the differences should be the greatest of all recorded. Eight scaffold weights at time = 0 weeks were pooled and compared using ANOVA to their weights at time = 10 weeks (Fig. 5). There was a significant drop in weight, but the drop was small, resulting in a weight reduction of nearly 4% of the scaffolds average starting weight.

Mechanical testing was conducted in compression at a displacement rate of 1.27 mm/min using a Mark-10 electromechanical testing machine (Copiague, NY) (Fig. 4). Compression test were made between steel plates and specimens were only loaded within the elastic region of the material. Following testing the structural stiffness (Load/displacement) was calculated form the slope of the load-displacement curve within the linear elastic region. Statistical analysis using JMP (SAS institute, Cary, NC) was performed to detect degradation in weight and stiffness with soak time. A significant difference is indicated by P <0.05.

Osteoblast Attachment on PLA
A total of eight PLA specimens were placed in the cell culture protocol. Four of the specimens were cultured with fibroblast as a control and four were cultured with large T transfected human osteoblasts as the test group according to the following protocols. After printing, the X scaffolds were sterilized in 70% isopropl alcohol for 24 hours then air-dried in a HEPA filtered BL-2 Biosafety cabinet. The last step in sterilization included a two hour UV irradiation in the biosafety cabinet. At the time of plating and co-incubation with sterile scaffolds, cells were viable and proliferating. For both cell lines throughout the study, all recommendations for culturing and passaging of large T antigen transfected human osteoblasts (CRL-11372) by the ATCC and normal human fibroblasts were followed. Individual 3D printed scaffolds were placed in a 12-well plate with five mL of complete growth media, and 5,000 cells/cm2 then incubated at 37 °C with new media added every two days. After four days of culture the scaffolds were removed from media, fixed in freshly prepared 2% PBS-buffered paraformaldehyde for 15 minutes at room temperature. Scaffolds with attached cells now fixed to the scaffold were rinsed with PBS twice, for five minutes. To assess cell attachment scaffolds were submerged in trypan blue solution, which binds cell structures and allows visualization under light microscopy, then rinsed with PBS for five minutes.

Discussion
The first objective of this project was to determine if PLA scaffolds degrade with time while soaking in cell culture media. Results indicated that although minimal weight loss occurs, it appears as though PLA scaffolds tend to get stiffer with soaking. This observed behavior is not fully understood at this time. The second objective of this project was to observe if bone precursor cells (osteoblasts) could attach and survive on PLA. Microscopic evidence showed that large T antigens transfected human osteoblasts attached and survived on 3D printed PLA scaffolds. The frequency and locations mimicked that of fibroblasts with is known to survive and proliferate on PLA.

References

Acknowledgement
The authors would like to acknowledge Ohio Space Grant Consortium for funding. They would also like to thank Dr. Robert Chasnov for administering this program at Cedarville University and helping to make this work possible.