3D-Bioprinted PCL/GelMA-HA Scaffolds for Guided Bone Regeneration (GBR)

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INTRODUCTION

Periodontitis is a prolific, inflammatory gum disease that can eventually lead to tooth loss if untreated. Researchers are striving to create an ideal implantable device that will regenerate the lost tissue and ligament of the periodontium. Such an implant must be biocompatible, bioresorbable and bioactive, so that it can facilitate cell growth and proliferation. Polycaprolactone (PCL) scaffolds were 3D-printed and treated with non-equilibrium low-temperature plasma (LTP). Gelatin-methacryloyl (GelMA) and a GelMA-hydroxyapatite (HA) hydrogels were fabricated and optimized before being infiltrated into our scaffolds.

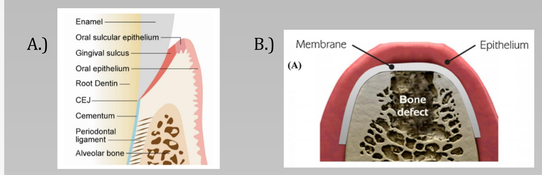


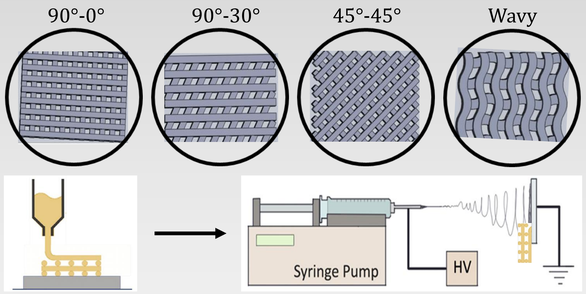
Figure 1: Diagram of periodontium, healthy (A) and unhealthy with membrane implant (B).

Objective: Synthesize a biocompatible, bioresorbable, bioactive membrane, infiltrated with hydrogel, to fill the space of the defect and regenerate the surrounding tissue.

This study compares the effects of non-equilibrium, low-temperature plasma (LTP) treatment on the hydrophilicity, mechanical strength and cell viability of four scaffolds of varying design, while also exploring the infiltration success and properties of gelatin-methacrylol (GelMA) and GelMA-hydroxyapatite (HA) hydrogels.

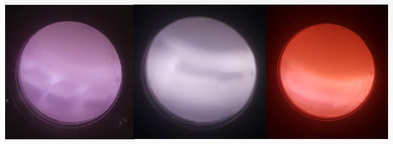
MATERIALS & METHODS

1. Fabrication of Scaffolds



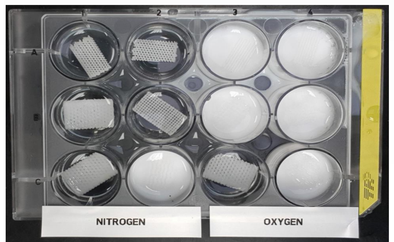
Four scaffold designs were created using SolidWorks CAD software. Polycaprolactone (PCL), a viscoelastic polyester, was 3D-printed with a Fused Deposition Modeling (FDM) MakerBot printer. A thin layer of PCL was then electrospun on top of the printed scaffold using a 20% w/v solution of PCL/HFIP.

2. Plasma Treatment of Scaffolds



Scaffolds were treated with non-equilibrium, low-temperature plasma (LTP), using N_2, O_2 and H_2 gases shown from left to right.

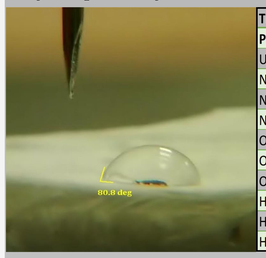
3. Fabrication & Infiltration of Hydrogels



Photoinitiator Irgacure 2959 was added to GelMA and GelMA-hydroxyapatite (HA) at 1.5% w/v concentration and crosslinked for 3 minutes with a wavelength of 365nm.

RESULTS & DISCUSSION

Hydrophilicity



Time after drop:	1s	10s	30s
Plasma		Contact Angle (°)	
Untreated	80.8 ± 2.0	77.9 ± 2.0	72.2 ± 2.0
N 10s	27.8 ± 2.0	0	0
N 20s	0	0	0
N30s	0	0	0
O 10s	0	0	0
O 20s	0	0	0
O 30s	0	0	0
H 10s	82.2 ± 2.0	78.2 ± 2.0	76.1 ± 2.0
H 20s	0	0	0
H 30s	N/A	N/A	52.8 ± 2.0

LTP was shown to significantly increase hydrophilicity of the PCL scaffolds via water contact angle.

X-Ray Photoelectron Spectroscopy (XPS)

Plasma Treatment	Surface Element	Relative Percentage (%)
None (Control)	C	74.3
	O	**19.5**
Nitrogen 6min	C	62.8
	O	**35.9**
Nitrogen 10min	C	56.4
	O	**36.1**
Oxygen 2min	C	49.9
	O	**38.8**
Oxygen 3min	C	67.3
	O	**24**
	N	1.7
Oxygen 6min	C	53
	O	**27.9**
	O	26.3
Hydrogen 6min	C	58.6
	O	**26.3**
Hydrogen 10min	C	57.7
	O	**27.6**
	N	3.2

Figure 2: XPS data comparing relative atomic percentages of Carbon, Oxygen and Nitrogen on PCL scaffolds after varying plasma treatments. O_2 plasma treatment created the highest relative percentage of oxygen on the surface. O_2 plasma also has the ability to physically etch the surface of the treated material; this was observed for treatment times of 6+ minutes.

LTP was performed under 45W radio frequency (RF) with a flow rate of ~15sccm and a pressure of ~500mTorr.

Mechanical Testing

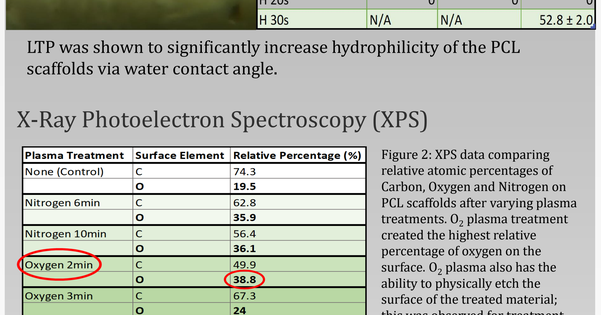


Figure 3: Graphical data showing the optimal combination of 1.5% photoinitiator (PI) and a crosslinking time of 3 minutes for hydrogel.

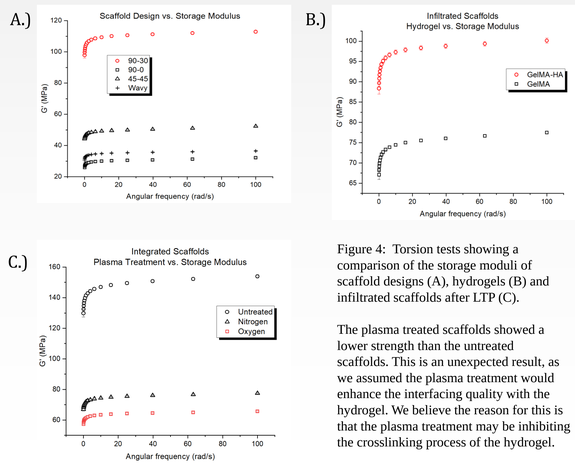


Figure 4: Torsion tests showing a comparison of the storage moduli of scaffold designs (A), hydrogels (B) and infiltrated scaffolds after LTP (C).

The plasma treated scaffolds showed a lower strength than the untreated scaffolds. This is an unexpected result, as we assumed the plasma treatment would enhance the interfacing quality with the hydrogel. We believe the reason for this is that the plasma treatment may be inhibiting the crosslinking process of the hydrogel.

RESULTS & DISCUSSION

Scanning Electron Microscopy (SEM) Imaging

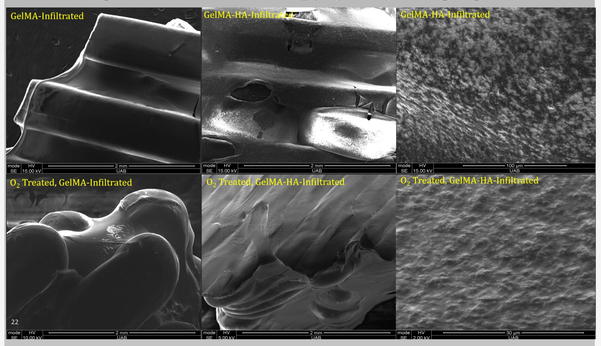


Figure 5: Comparison of hydrogel infiltration before and after LTP.

It appears as though the O_2 treated scaffold resulted in a more successful, evenly coated infiltration, specifically with the GelMA-HA hydrogel.

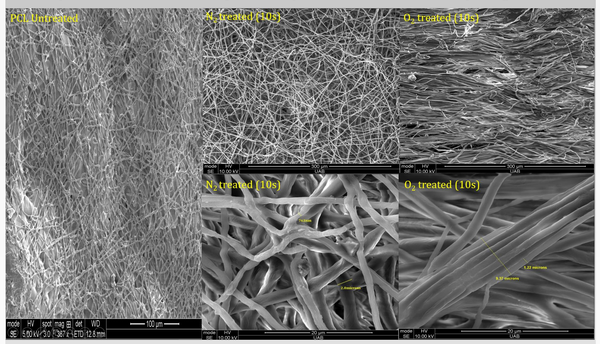


Figure 6: Comparison of electrospun nanofibers before and after LTP.

There is a noticeable difference in the structure of the fibers that have been treated with O_2 plasma. This may offer a way to better mimic the fibril bundle nature of the extra-cellular matrix (ECM).

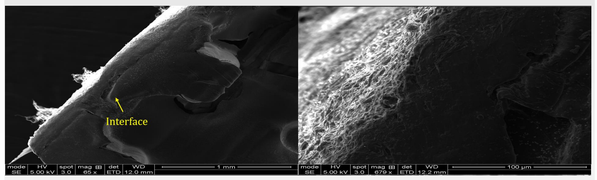


Figure 7: Interface between electrospun and 3D-printed layers in bilayer scaffold.

Cell Viability

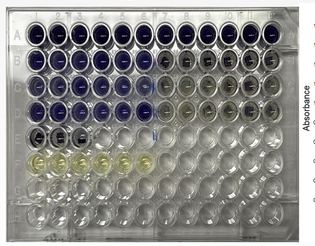


Figure 8: 96-well plate in which the samples were loaded and MTT assay was performed. A1-A12: plated cells for a control. B1-B6: GelMA infiltrated, untreated scaffolds. B7-B12: GelMA-HA infiltrated, untreated scaffolds. C1-C6: GelMA infiltrated, N_2 treated scaffolds. C7-C12: GelMA-HA infiltrated, N_2 treated scaffolds. D1-6: GelMA infiltrated, O_2 treated scaffolds. D7-12: GelMA-HA infiltrated, O_2 treated scaffolds. E1-E3: untreated PCL scaffolds with no hydrogel infiltration. F1-F6: blank wells for control.

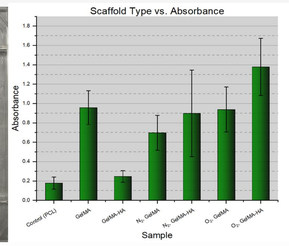


Figure 9: Graphical representation of the MTT assay showing the absorbance of each type of sample.

Upon visual analysis of Figure 8, it appears as though the GelMA-HA infiltrated samples did not perform as well as the GelMA infiltrated samples, despite HA's ability to enhance bone cell growth. This is certainly an unexpected result. The reason for this may be attributed to the concentration of HA used in the formation of the hydrogel (5% w/v), or possibly the method of integration of HA with the hydrogel. These things should be further explored. Additionally, there is a discrepancy in this data when analyzing Figure 9. We believe this may be due to residual HA in the wells, which was absorbing light after removing the samples. Finally, it appears as though there might be a decrease in cell viability with the plasma treated samples versus the untreated ones. Cellular studies are inconclusive and will be repeated.

CONCLUSIONS & FUTURE STUDY

- ❑ To the best of our knowledge, we have produced a unique type of membrane for GBR that has not been produced before.
- ❑ Unexpectedly, LTP had adverse effects on the mechanical integrity of the infiltrated scaffolds as compared to the untreated ones, which was a surprising result that poses interesting questions regarding the effects of LTP on photopolymerization.
- ❑ Through SEM, it appears that LTP (specifically with O_2) may enhance the effectiveness of scaffold infiltration with GelMA and GelMA-HA hydrogels.
- ❑ Effects of LTP on the crosslinking process should be further investigated.
  - Degree of crosslinking should be measured after plasma treatment.
  - Mechanical strength of treated scaffolds should be tested both before and after infiltration.
- ❑ This is an ongoing project with many of these experiments being repeated, especially the cellular study.

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