Quantitative Analysis of Histological PCL Scaffolds Stainings

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Introduction

Tissue engineering is a discipline in which scaffolds, cells, & other biological molecules are constructed and utilized to "restore, maintain, or improve damaged tissues" (8).

This project specifically focuses on the implantation of 3D-printed poly ε-caprolactone (PCL) scaffolds into nude mice. Having already fabricated them prior to the beginning of this semester, Dr. Freeman implanted dozens of scaffolds into nude mice samples to investigate how different scaffold structure would affect vessel infiltration (4). Half of the scaffolds were implanted for two weeks and the other half were implanted for eight weeks. For each of the four scaffold geometry at each of the two time periods, there were four measures of average vessel counts, totalling 32 data points.

Research Aims

- Quantify vessels across different regions of the scaffold samples
- Compare the implantation time of the scaffolds (two or eight weeks) and four different geometries of the scaffolds (Geo 1, Geo 2, Geo 3, and Geo 4) to vessel count

Methodology

- Histological analysis was conducted on the scaffold sections using Hematoxylin and Eosin (H&E) staining. These were then put into a bioimage analysis software, QuPath, to undergo the counting process
- Data and statistical analysis were then performed using a statistics software, Prism 10 from GraphPad

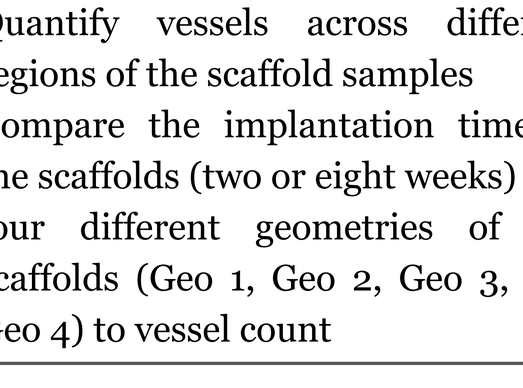

Figure 1: Sample Vessel Quantification. This figure is a screenshot from QuPath, where two vessels are identified.

Acknowledgements

I want to give a special thank you to Dr. Freeman and Dr. Nolan for their helpful guidance throughout this semester.

Results

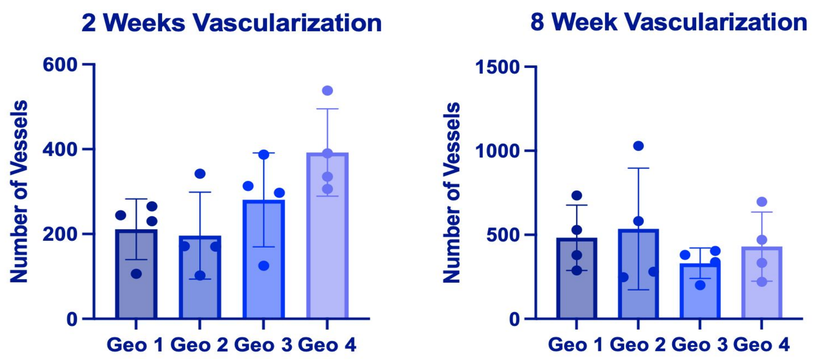

Figure 2: Vessel Counts per Geometry with all Data Points. These bar charts represent the average vessel counts per scaffold section with standard deviation across the two time periods and four scaffold geometries.

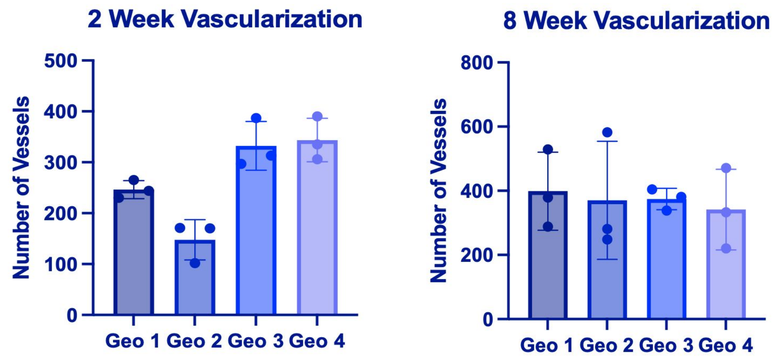

Figure 3: Vessel Counts, Excluding Outliers

Table 1: One Way ANOVA Testing for Two Week Implantation Period, Excluding Outliers. This table shows that there is statistical significance between Geo 2 and Geos 3 and 4 (Since $P < 0.05$), showing scaffold geometry may have influence on vessel growth

Tukey's Comparison Test	Mean Diff.	Below threshold?	Summary	Adjusted P-Value
Geo 1 vs. Geo 2	98.67	No	ns	0.0561
Geo 1 vs. Geo 3	-86.00	No	ns	0.0988
Geo 1 vs. Geo 4	-97.33	No	ns	0.0596
Geo 2 vs. Geo 3	-184.7	Yes	**	0.0017
Geo 2 vs. Geo 4	-196.0	Yes	**	0.0012
Geo 3 vs. Geo 4	-11.33	No	ns	0.9831

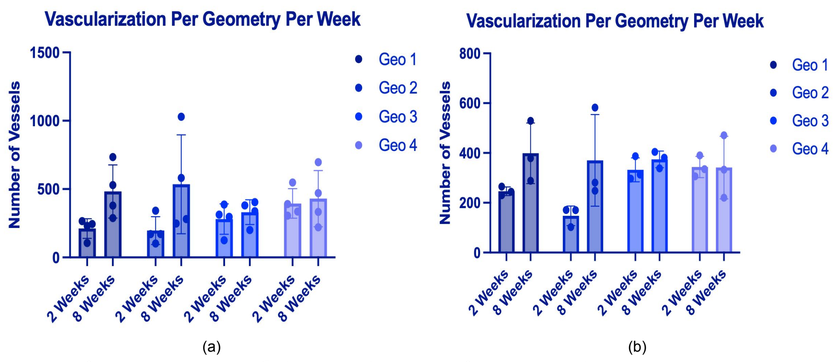

Figure 4: Vessel Count Comparison Per Geometry Per Implantation Period. Figure 3a shows the vascularization with all data points, and Figure 3b shows the vascularization excluding outliers.

Table 2: Two Way ANOVA Testing, Including All Data Points. This table shows that scaffold implantation time has the only statistically significant effect on vessel growth, with a P-Value of 0.0047

Source of Variation	% of Total Variation	P-Value	Summary	Significant?
Time x Geometry	11.76	0.1252	ns	No
Time	20.04	0.0047	**	Yes
Geometry	3.894	0.7782	ns	No
Subject	44.19	0.0937	ns	No

Table 3: Two Way ANOVA Testing, Excluding Outliers

Source of Variation	% of Total Variation	P-Value	Summary	Significant?
Time x Geometry	16.49	0.2223	ns	No
Time	22.46	0.0261	*	Yes
Geometry	11.17	0.3830	ns	No
Subject	25.66	0.4685	ns	No

Discussion

One Way ANOVA Testing:

- Most of the cross-geometry comparisons showed that scaffold geometry does not impact vascularization, apart from:
  - 2 week scaffold implantation, excluding outliers, where Geo 2 and Geo 3 had a P-Value of 0.0017 and Geo 2 and Geo 4 had a P-Value of 0.0012.

Two Way ANOVA Testing:

- With and without outliers, the correlation that measured most statistical significance with vessel count was scaffold implantation time, having measured P-Values of 0.0047 and 0.0261, respectively. This is defended by previous research, in which longer implantation time results in further angiogenesis (1).

Conclusion

- Apart from one trial (2 week implantation, excluding outliers), there was generally no strong correlation between the individual four scaffold geometries and vessel growth found, with and without outliers.
- The most statistically significant measure when compared to vascularization was that of implantation time.
- For future experiments, more data points should be taken and quantified to give stronger, more justifiable, and more believable statistical conclusions.

References

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