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Chitosan Membranes for Biodegradable Microfluidics

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Clinical Need

Angiogenesis is a vital requirement for the formation of healthy tissue and bone. Currently this is the greatest challenge in the field of tissue engineering. A promising strategy to achieve formation of suitable, intrinsic vasculature is the use of biodegradable microfluidic networks.

Biodegradable scaffolds may be constructed by a **numbering up** approach where several layers of microfluidic medical devices are compiled together via a multi-scale design. The micro-channels increase cell proliferation aiding in improved attachment between implants and biological material. The enhanced capillary forces will allow for the circulation of the nutritional components which are necessary for the growth of cells.

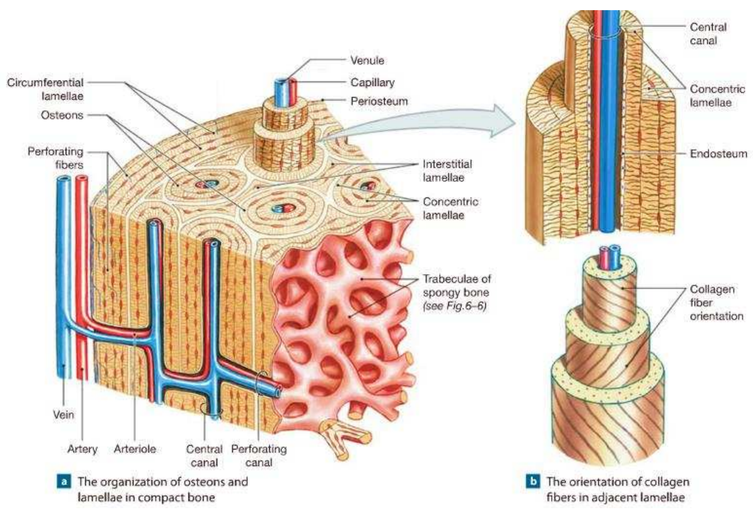


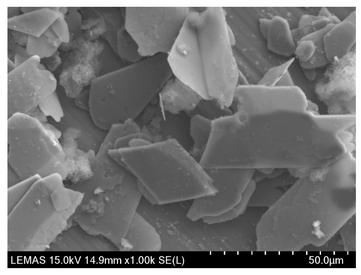
FIGURE 1. Blood vessels in bone

Aim

Use a femtosecond laser to create microchannel networks upon chitosan membrane surfaces.

Methods & Materials

Brushite Mineral Preparation



$CaHPO_4 \cdot 2(H_2O)$

Membranes Preparation

TABLE 1. Created membranes

Membrane	Codification	Brushite (g)	Erbium (g)	Appearance
Pure Chitosan	C1gT	0	0	Pale yellow colour
Brushite-Chitosan	B0.5gg	0.5	0	Opaque colour
Brushite-Chitosan	B1gT	1	0	Opaque colour
Erbium-Chitosan	E0.5gT	0	0.5	Pink colour
Erbium-Chitosan	E1gT	0	1	Pink colour

- A solution casting method was used to create pure chitosan and mineral membranes.
- Chitosan flakes were slowly mixed with acetic acid and distilled water.
- Next mineral (brushite/erbium) was added.
- The solution was covered and left mixing for 24hrs at 25ºC.
- The solution was cast into dishes and dried at 50°C for 24hrs thus forming membranes.

Mechanical Testing

Ultimate Strength Testing

Three samples were mechanically tested from each non-treated and NaOH treated membranes and the equipment parameters used:

- 10mm Jaw Separation
- 100N Load Cell
- 100mm/min Speed
- 3.5 X 0.5 cm Sample Dimension
- Tests performed at room temperature

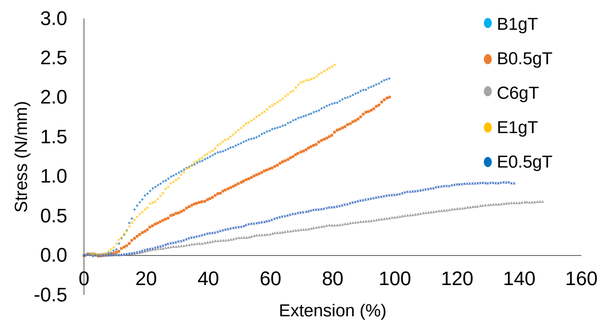


FIGURE 10. Plot of stress against extension for all membrane samples.

- 1g erbium sample has highest strength but lowest extension
- 0.5g erbium sample has almost similar extension to the pure chitosan sample.
- Both brushite samples has similar strengths and extensions.

Femtosecond Laser

Channel Formation

A Femtosecond Laser Micromachining System, LIBRA-S-1K by COHERENT was used. The equipment and lens parameters used for the creation of microchannels upon the membrane surfaces included:

- 800nm Wavelength
- 1kHz Repetition Rate
- 80-100f s Pulse Duration
- 5.5mm Working Distance
- 20X Lens

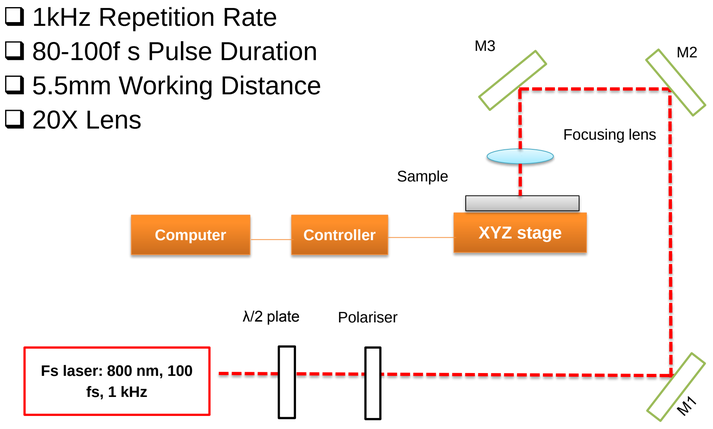


FIGURE 2. Experimental Setup

Microchannels

- Increase in power also caused an increase in channel width (figure 3).
- Brushite channel widths were larger compared to Erbium membrane samples.
- Creation of microchannels upon membrane surfaces were successful.
- There was no damage to any of the surrounding areas (figure 4).

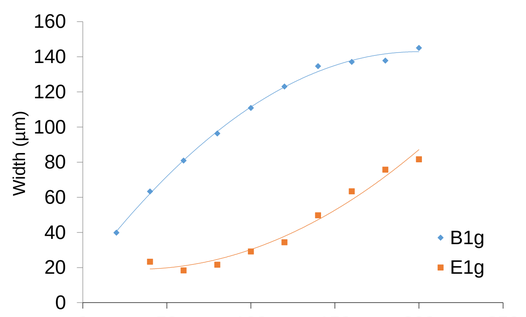


FIGURE 3. Chitosan-1g brushite average channel widths

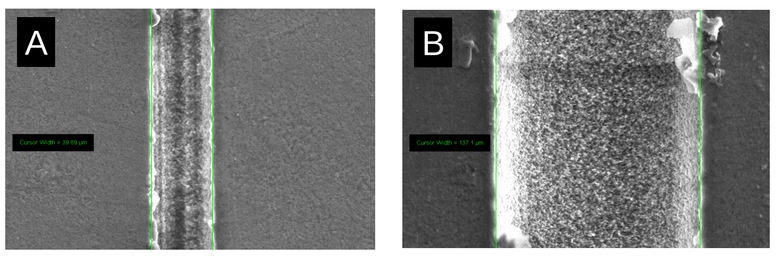


FIGURE 4. (A) 39.89µm chitosan-1g brushite channel width, (B) 137.1µm chitosan-1g brushite channel width

Fourier Transform Infrared

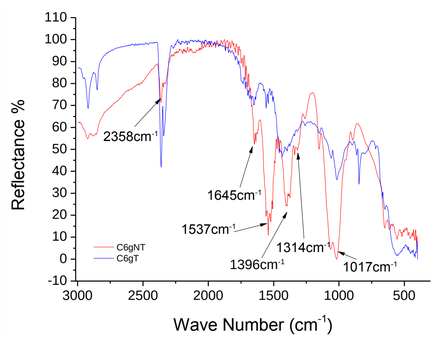


FIGURE 5. NaOH treated and untreated pure chitosan samples.

TABLE 2. Bond Assignments

Wavenumber, cm^{-1}	Assignments
2919	O-H and N-H bonds
2356	CH_2 symmetric/asymmetric pyranose ring vibrations.
1653	C=O bond (amide I)
1559	N-H bond (amide II)
1435	CH_3 deformation mode
1396	CH_3 deformation mode
1315	amide III bond
1017	C-O bond stretching
846-1156	Saccharide structure

Micro patterns

- Femtosecond pulsed laser may also be used to create various patterns.
- Figures 6 and 7 depict close up of crisscross patterns ablated upon chitosan-1g brushite membranes.

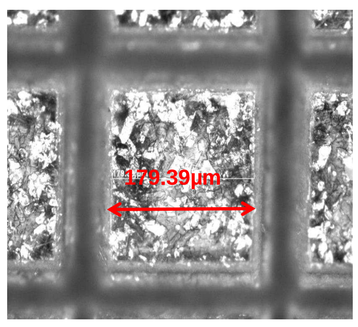


FIGURE 6. Chitosan-1g brushite average channel widths.

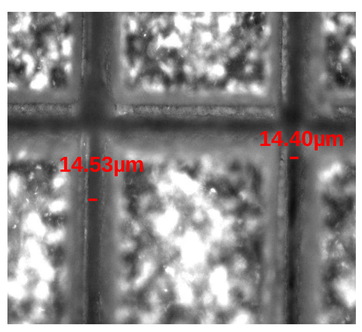


FIGURE 7. Chitosan-1g brushite average channel widths.

Ablation Threshold

- Ablation threshold varies between different materials; initial ablation testing has been demonstrated for membranes containing brushite.
- Each spot was created using 3000 pulses in a space of 3 seconds.
- Squared diameter of the ablation spots against the laser energy can be used to measure ablation threshold (figure 8).

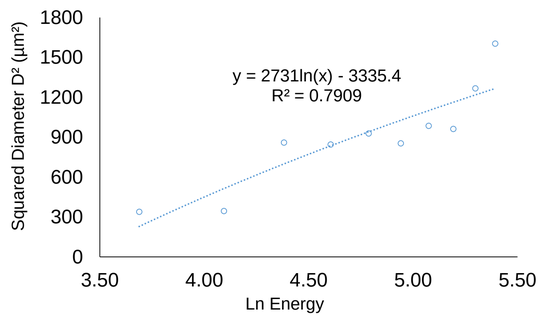


FIGURE 8. Squared diameter of ablation spots in chitosan- 1g brushite membrane

Degradation Results

Membranes were placed weekly in fresh buffered saline solution consisting of:

- 0.01 M phosphate buffer
- 0.0027 M KCl
- 0.137 M NaCl
- pH of 7.4

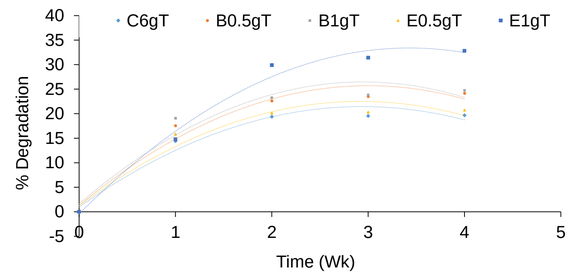


FIGURE 11. Degradation Results.

- A consistent degradation trend was established during the 4 week testing (figure 11).
- Although the results have been promising, long term testing still needs to be investigated to determine the rate of degradation
- Further characterisation of materials after degradation has occurred needs to be investigated.

The same buffered saline solution was used to determine the amount of water uptake in relation to mass increase as shown in figure 12.

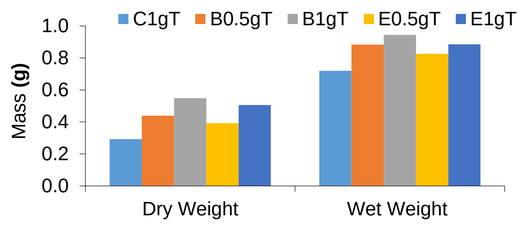


FIGURE 12. Plot of mass increase with water uptake.

Water content (W.C) = $(W_W - W_d) / W_W$

Where Ww and Wd are the weight and dry weights of the membrane

Conclusion

- Incorporation of mineral enabled for improvement of mechanical strength.
- Degradation results indicate the membranes suitability for creation of potential scaffolds aiding in the regeneration of bone.
- Using a femtosecond laser we managed to create suitable microchannels without damaging the surrounding material.
- Channel width size created ranged between 30-140µm depending upon the laser power used.

Future Work

- Cell viability and toxicity testing
- Construction of multi-layered scaffold

References

[1] Leceta, I., et al., Characterization and antimicrobial analysis of chitosan-based films. Journal of Food Engineering, 2013. **116**(4): p. 889-99.

[2] Wang, J., et al. Biodegradable microfluidic scaffolds with tunable degradation properties from amino alcohol-based poly(ester amide) elastomers. in 2010 MRS Fall Meeting, November 29, 2010 - December 3, 2010. 2011. Boston, MA, United states: Materials Research Society.