Biosynthesized cellulose for use in a novel drug delivery system to treat the lingering effects of stroke

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1. Introduction and Background

Stroke is one of the leading causes of death and disability in Canada; statistics show that nearly 14,000 Canadians die from stroke every year.[1] Although the number of survivors continues to grow thanks to advances in modern medicine, many patients are left with irreparable conditions that can only be alleviated by rehabilitation. No treatment is available for these survivors and rehabilitation costs thousands of dollars to the patients and their families.

Strokes are events that prevent the neurons in the brain from receiving oxygen from the bloodstream and as such cause cell damage and cell death. Unlike other cell types in the human body, neurons have a very limited capacity for regeneration and that quality prevents many victims from making a full recovery. The need for new methods of treatment has never been greater, and recent scientific developments may provide the key to save countless lives.

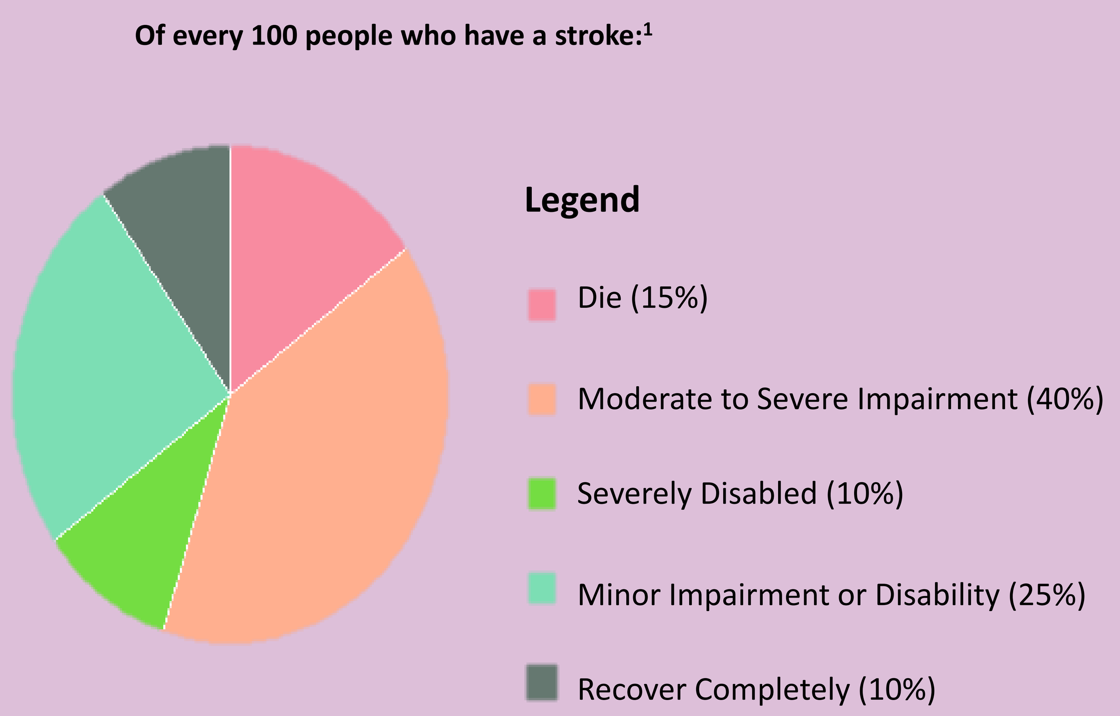

Figure 1. A pie chart demonstrating the statistics surrounding the long term effects of stroke and how few people fully recover.

The usage of biosynthesized cellulose (BC), a hydrogel that has shown considerable biocompatibility[2], may provide a useful medium through which drugs may be delivered to treat the crippling effects of stroke.

BC is a hydrogel produced by many different species of bacteria. Hydrogels are dense networks of interconnected fibers which form a porous structure capable of absorbing significant quantities of the surrounding solution. This quality paired with its ability to bind to human cells makes it a promising candidate for a controlled drug delivery system.[3]

2. Methodology

2.1 Biosynthesis of BC

The media used to supply nutrients to the bacteria was prepared by mixing 20.0 g of glucose, 5.0 g of peptone, 5.0 g of yeast extract, 2.7 g of disodium phosphate, 1.5 g of citric acid in 1 L of distilled water. The mixture was then autoclaved at 121° C for 20 minutes.

Gluconacetobacter hansenii was inoculated in the media and left to incubate at 26° C for 1.5 weeks. The collected membranes were washed overnight in a solution of NaOH at 50° C and rinsed several times with distilled water until pH 7.

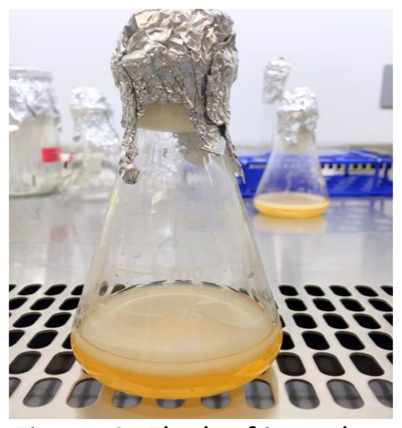

Figure 2. Flask of inoculum containing a BC membrane.

2.2 Production of blended BC membranes

Never-dried BC membranes were manually compressed to remove as much water as possible, weighed and blended using 70 mL of distilled water until the suspension of cellulose pulp contained no dense pieces. The suspension was then passed through a fritted Buchner filter of small porosity to maximize the removal of water while minimizing the loss of cellulose. The membranes of blended BC (BBC) were removed from the filter once no gel was visible.

Figure 3. BBC gel before filtration.

2.3 Testing

The BBC membranes were separated into 5 groups based on their mass of cellulose in order to test their properties. BBC membranes were weighed, frozen, lyophilized and then weighed again in order to determine the overall cellulose content and the water holding capacity. The lyophilized membranes were submerged into water and weighed to test their rehydration ratio after 5 minutes, 30 minutes, 1 hour, 2 hours, 24 hours and 48 hours.
The rehydrated BBC membranes had their tensile strength, elongation and Young's modulus measured in an Instron electrodynamic test instrument. Rectangular BBC membranes were cut (15 × 10 × 0.65 mm) and stretched lengthwise; the crosshead speed was set at 5 mm·min^{-1}. All tests were performed at least in triplicate.

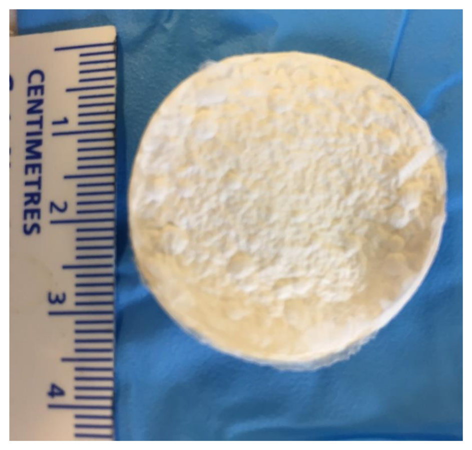

Figure 4. BBC membranes after lyophilisation.

3. Results

Both the rehydration and tensile strength tests provided useful results that help explain the properties of the BBC membranes. The membranes demonstrated a relatively constant capacity for water absorption in relation to the percentage of cellulose, the average value of which being 85.49 %. Despite this constant, the rehydration tests demonstrate the group containing the membranes with the lowest masses of cellulose present the greatest capacity for rehydration, reaching a rehydration ratio of 78.89 % which is 12.59 % greater than the group of highest mass.

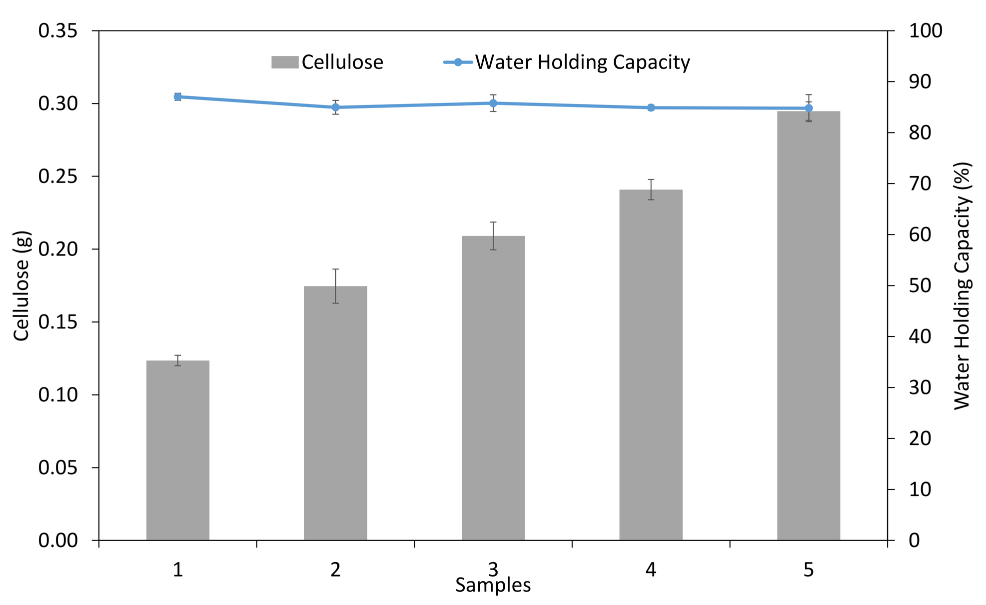

Figure 5. Water holding capacity in relation to the amount of cellulose present in the BBC membranes.

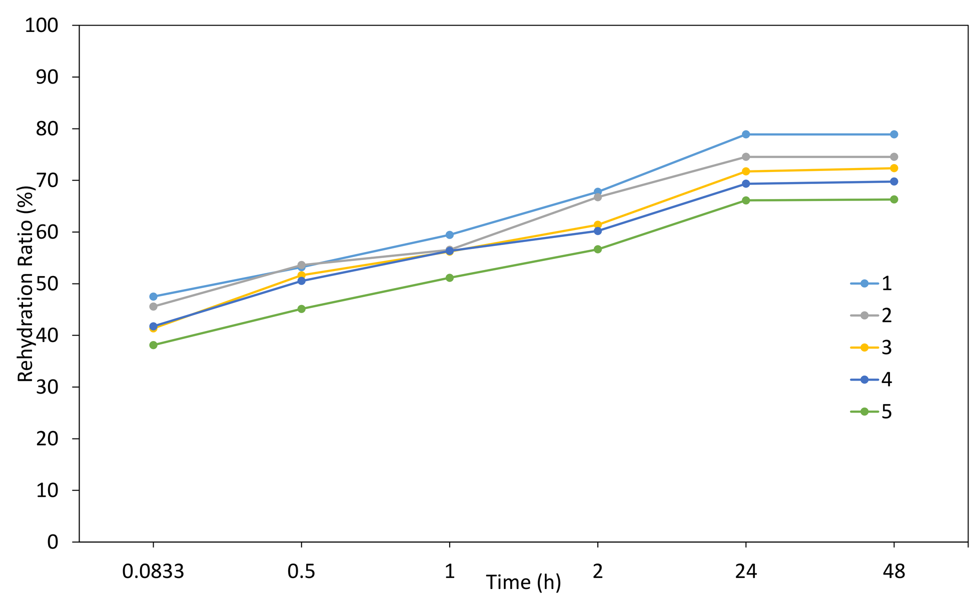

Figure 6. Rehydration ratio over time of the different groups of BBC membranes separated in ascending order by my mass of cellulose.

Furthermore, the membranes acted in reverse order during the mechanical tests. The BBC membranes containing the most cellulose and the least amount of water per weight (group 5) demonstrated greater elasticity and tensile strength when compared to the membranes of lesser mass and greater rehydration ratio (groups 1-4).

Table 1. Cellulose content and mechanical properties of BBC membranes.

Sample	Cellulose (g)	Young's Modulus (MPa)	Ultimate Tensile Strength (MPa)	Elongation-at-break (%)
1	0.1235 ± 0.004	0.047 ± 0.02	0.175 ± 0.05	3.982 ± 0.63
2	0.1712 ± 0.006	0.108 ± 0.01	0.317 ± 0.15	4.342 ± 0.43
3	0.2091 ± 0.007	0.151 ± 0.01	0.516 ± 0.07	4.051 ± 0.56
4	0.2409 ± 0.006	0.190 ± 0.03	0.698 ± 0.06	4.411 ± 0.29
5	0.2948 ± 0.029	0.365 ± 0.02	0.964 ± 0.04	3.150 ± 0.04

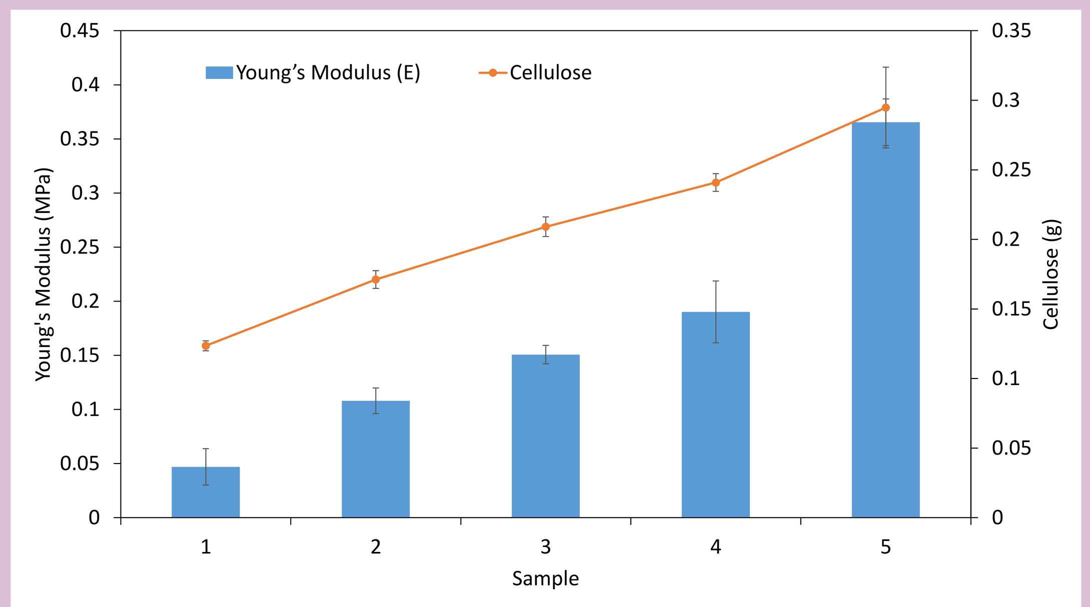

Figure 7. Young's Modulus in relation to the amount of cellulose found in the BBC membranes.

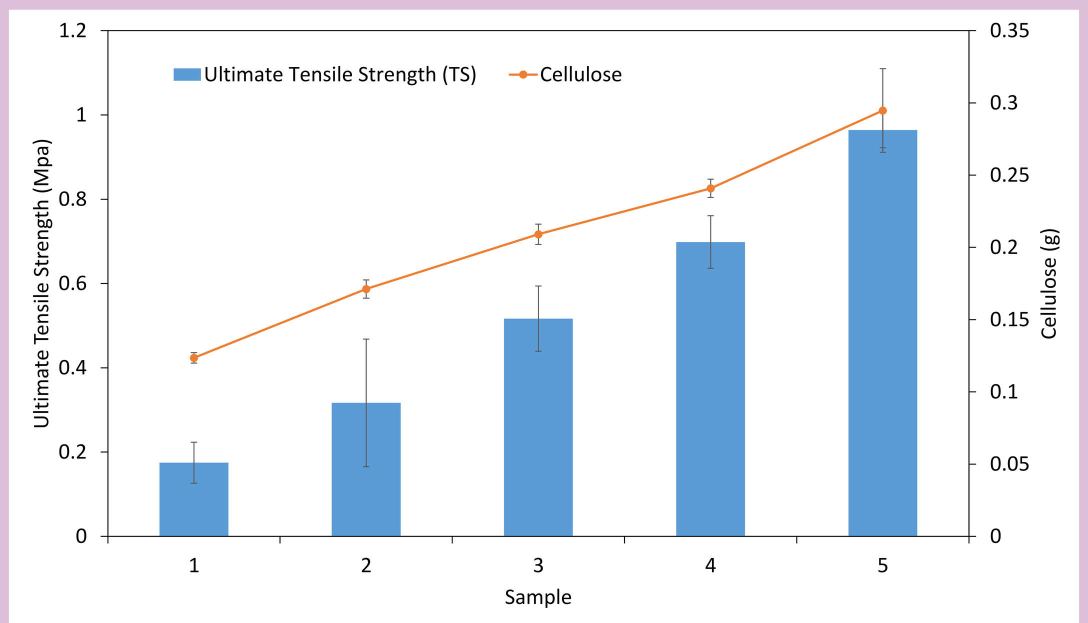

Figure 8. Ultimate tensile strength in relation to the amount of cellulose found in the BBC membranes.

4. Conclusion

The BBC membranes were successfully produced by blending membranes originating from the bacterial cultures. The BBC membranes containing the most cellulose showed a greater resilience to physical strain with a Young's Modulus of 0.3654 MPa and a ultimate tensile strength of 0.9642 MPa all while boasting a rehydration ratio of 66.30 %. Properties which were expected and are necessary in the production of a stable scaffold for drug delivery; showing that these membranes are a viable candidate for such an application. Future studies include the integration of bovine serum albumin (BSA) as a substitute for cell growth factors in order to test the incorporation rate of the BSA into the membranes.

Acknowledgements

This project would not have been possible without the opportunity provided by the University of Ottawa's Office of Undergraduate Research and the support and guidance of my supervisors.

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