6-Bromoindirubin-3′-Oxime Incorporation in the Guanosine Diphosphate Crosslinked Chitosan Scaffold as a GSK3B Inhibitor: An Investigation of the Material Properties for Bone Regeneration

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Introduction

- Critical Size Bone Defects result from congenital conditions, tumor resections, and trauma[1]. To overcome treatment limitations, biological substitutes, designed based on the ideals of the diamond concept, are being explored at the defect site to aid in bone regeneration.
- Our laboratory has previously developed a guanosine diphosphate (GDP) cross-linked chitosan sponge[1,2], which gels rapidly allowing localization at the injury site and high entrapment efficiency[2]. The scaffold also has beneficial properties including cell compatibility and heterogenous pore sizes.
- The goal of this research is to design a new formulation of the GDP crosslinked chitosan sponge with the incorporation of 6-bromo-indirubin-3'-oxime (BIO). BIO is an indirubin derivative that plays an essential role in bone formation through the promotion of mesenchymal progenitor cell differentiation[3,4]. The chemical and physical properties of this new scaffold are assessed as well as the scaffold's cellular compatibility and ability to promote differentiation.

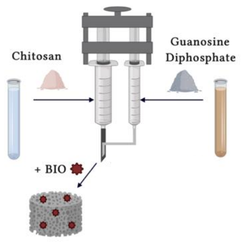
Objectives

- Confirmation of BIO incorporation into the scaffold using quantitative and qualitative methods
- Characterization of this new scaffold's material properties
- Assessment of cellular biocompatibility and distribution

Materials and Methodology

Guanosine Diphosphate
Anionic Crosslinker
Less Cytotoxic and improved cell compatibility

Chitosan
Promising biomaterial for tissue regeneration
Biocompatible, readily available



BIO Incorporation - Solid State 13C NMR, FTIR, visual assessment

Material Characterization - Gelation Time, Degradation, Rheology, SEM Imaging, MicroCT

Cellular Biocompatibility and Distribution - Alamar Blue, Immunofluorescent Staining, Live/Dead Cytotoxicity Assay

Results

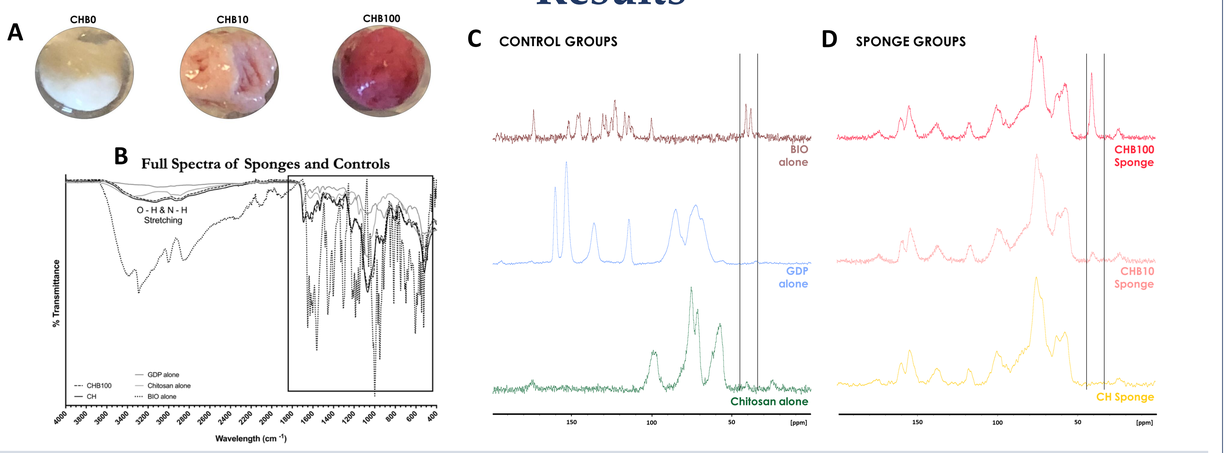


Figure 1. Incorporation of BIO in the Sponges. (A) Imaging of sponges showing differences in color among the concentrations. (B) FTIR spectra of control groups (BIO, GDP, chitosan and GDP Chitosan scaffold with and without BIO. (C,D) Solid State ^{13}C NMR spectra of sponges with increasing BIO concentrations (0 and 100 uM) along with controls (BIO, GDP, Chitosan).

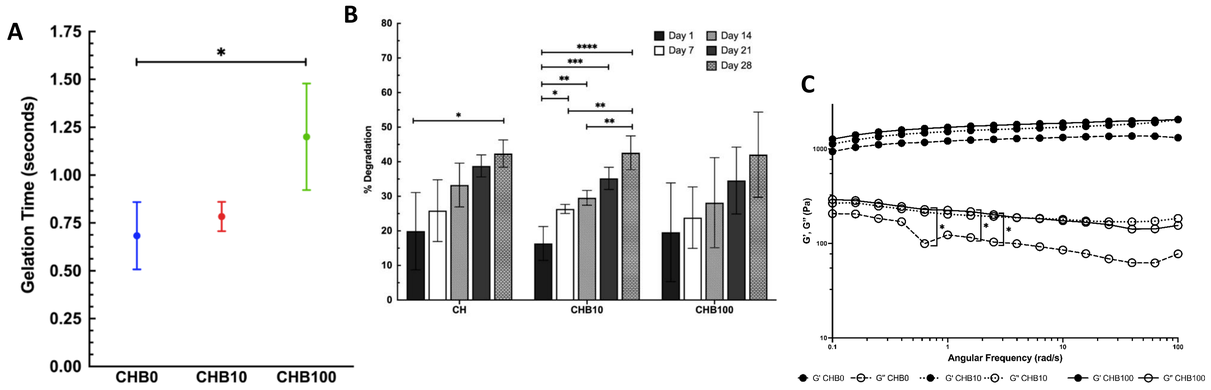


Figure 2. Material Properties of the Sponge. (A) Average gelation time measurements with increasing BIO concentrations. (B) Degradation profile of the BIO-incorporated sponges over 28 days presented as the % of mass lost compared to initial weight. (C) Elastic (G') and Loss (G'') moduli at 1% strain for Chitosan sponges with increasing concentrations of BIO 0, 10, and 100 µM.

Results

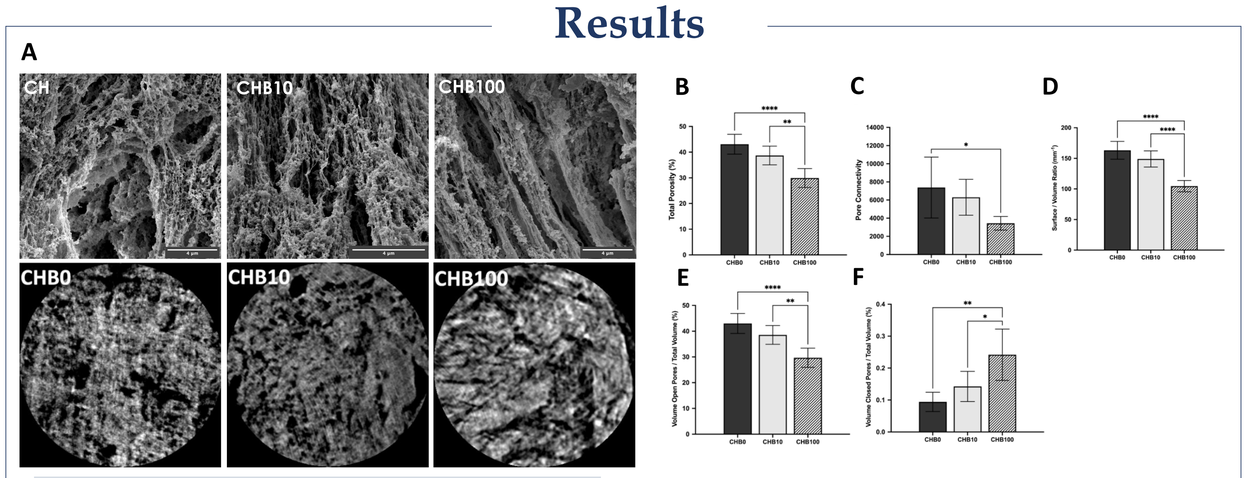


Figure 3. Internal Structure of the BIO Incorporated Scaffold. (A) SEM images and cross-sections of the internal structure of the BIO incorporated scaffolds (Diameter: 1 mm). Measurements from 3D-Analysis (Micro CT) for (B) total porosity, (C) pore connectivity, (D) surface to volume ratio, (E) % open pore volume/ total sponge volume, and (F) % closed pore volume/ total sponge volume.

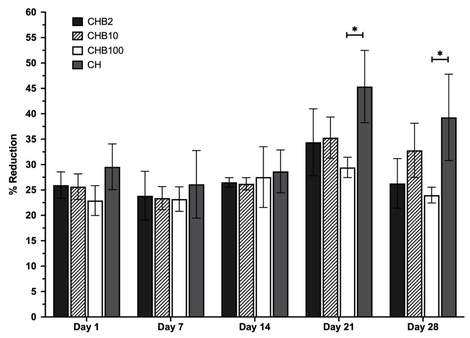


Figure 4. Cellular Metabolic Activity. Percent reduction of alamarBlue reagent of MC3T3 cells in chitosan scaffolds with varying BIO concentrations (0, 2, 10, 100 uM) over 28 days.

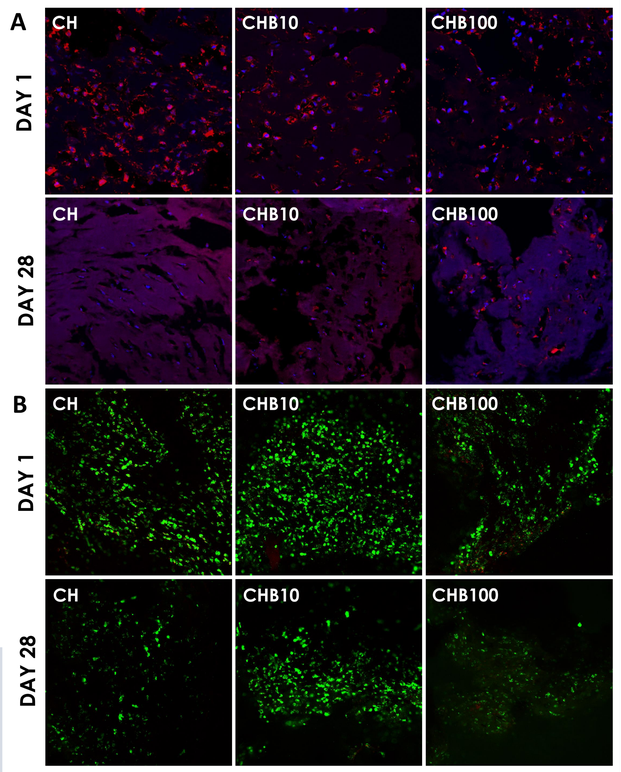


Figure 5. Cellular Biocompatibility and Distribution for MC3T3-E1 Cells at day 1 and 28. (A) cell distribution staining using Hoescht 33342 nucleic acid (blue) and iFluor 594 Phalloidin (red) staining in BIO incorporated chitosan sponges. (B) live/dead staining in BIO Incorporated Chitosan Sponges

Future Directions

- Examination of BIO's effect on ADSC differentiation in a cell stage specific manner
- Determination of optimal BIO doses for encapsulation *in vivo* in a mouse CSBD model

Conclusions

- BIO was successfully encapsulated in the GDP crosslinked chitosan sponge resulting in a new formulation of the sponge. This new formulation maintained a rapid gelation time (<1.6 sec), viscoelastic properties, and heterogeneous pore sizes regardless of the dosage of BIO.
- The BIO incorporated sponges supported the cells viability over 28 days with homogeneous cellular distribution.

Acknowledgements

The authors would like to acknowledge CIHR and NSERC for the financial support through the CHRP. The authors would also like to thank the lab members of Biomat'X Research Laboratories and the Willie Lab for their help. In addition, many thanks is offered to the McGill Chemistry Characterization Facility, the Facility for Electron Microscopy Research, the Advanced Bioimaging Facility and Professor Mongeau's Laboratory.

References

[1] L. Nayef, M. Mekhail, L. Benameur, J. S. Rendon, R. Hamdy and M. Tabrizian, *Acta biomaterialia*, 2016, **29**, 389-397. [2] M. Mekhail, J. Daoud, G. Almazan and M. Tabrizian, *Advanced healthcare materials*, 2013, **2**, 1126-1130. [3] J. Li, Z. Khavandgar, S.-H. Lin and M. Murshed, *Bone*, 2011, **48**, 321-331. [4] X.-E. Zhao, Z. Yang, Z. Gao, J. Ge, Q. Wei and B. Ma, *Anais da Academia Brasileira de Ciências*, 2019, **91**.