Characterization and application of a novel microsphere-laden bioink for generating stem cell-derived neural tissues

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INTRODUCTION

3D bioprinting allows the fabrication of complex architecture by layer-by-layer deposition of cells embedded in biomaterials. Our novel bioink consists of natural biomaterials such as alginate, fibrin, and genipin which is crosslinked with a crosslinker containing calcium chloride, chitosan, and thrombin. [1,3]Previous studies have validated that our microsphere-laden bioink can produce 3D neural tissue similar to the tissues found in human brain. However, the mechanical properties of the cellular microenvironment also have an important role in the morphogenesis of tissues as they influence both cellular and tissue compatibility. The purpose of this study was to determine the rheological properties of a bioink in order to understand the storage and loss moduli and establish the critical shear point and subsequent shear-thinning behavior for potential applications. In this study, the rheological behavior of our fibrin-based bioink was analyzed using a rotational rheometer, and an indentation method(4). The second goal of this study is also to understand how the addition of cells influences the rheological properties and printability of a bioink. This is important to know if these cells survive and function after the bioprinting process. The cell seeding density also affects the rheology and printability of bioink. Here, we also analyzed the bioprinted tissues with several different concentrations of neural progenitor cells.

(A) **(B)**

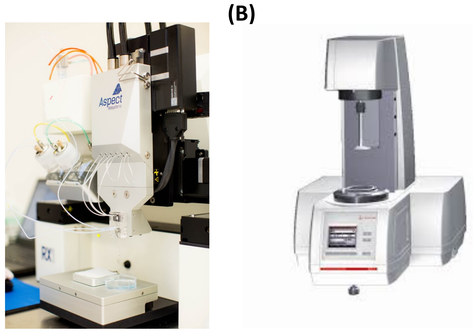

Figure1: (A) The Aspect Biosystems' RX1 bioprinter **(B)** The Anton- PAAR Modular Compact Rheometer

MATERIALS & METHODS

Neural progenitor cells (NPC), at densities relevant to neural tissue (5 million cells per mL), were incorporated with the combination of drug-releasing microspheres (1mg/mL) into bioink. The aim was to choose a cell concentration that would result in a mechanically strong structure. For this purpose, we studied four different concentrations and chosen 5 million cells per mL. However, for higher concentrations, dark clusters started to form, and if these will make large clumps, this could also lead to necrosis due to diffusion limitation. Microspheres are spherical microparticles that can be used as drug carriers. The size of microspheres is in 1-1000µm range. Further, dome-shaped constructs of 6 layers each with a 0.2mm thickness and approximately 1 cm were printed with Aspect Biosystems' RX1 bioprinter(2). After printing, for the first 5 days, constructs were cultured in NPC expansion media with FGF8 and Purmorphamine, then switched to maturation media; Brain Phys supplemented with 1% P/S, dbcAMP, ascorbic acid, and growth factors such as BDNF, GDNF, TGF-β3 for 40 days.

	Treatment (1mg/ml of bioink)	Codes
1.	Purmorphamine + Guggulsterone	PG
2.	Guggulsterone + Retinoic Acid	GR
3.	Retinoic Acid + Purmorphamine	RP
4	No Microsphere	N

Table 1 : Treatment and codes of bioprinted constructs

Bioink's rheological properties after gelation were assessed with two different methods. The first procedure is by using a rheometer for measuring the stress-strain relationship to understand the deformation properties of a material. Another method that includes spherical indenters that are positioned on top of the bioink samples, generating an indentation depth that is then correlated with elastic modulus.

RESULTS & DISCUSSION

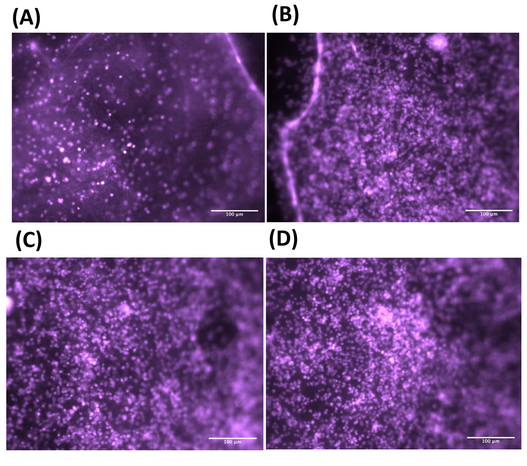

Figure 2: Fluorescent images of bioprinted construct showing comparison between four different concentrations of NPC (A) $2x10^6$/mL (B) $5x10^6$/mL (C) $7.5x10^6$/mL , and (D) 10^7/mL. Scale bars represent 100 µm.

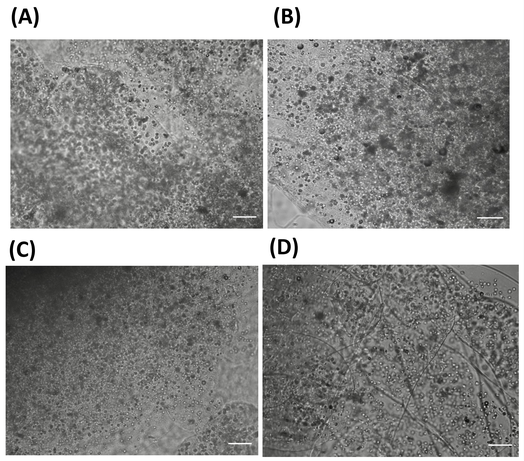

Figure 3: Phase contrast images of bioprinted constructs with 5 million cells/mL representing three different combinations of microspheres and no microspheres. (A) Guggulsterone + Purmorphamine
(B) Purmorphamine + Retinoic Acid, (C) Retinoic Acid + Guggulsterone, and (D) No Microspheres. Scale bars represent 100 µm.

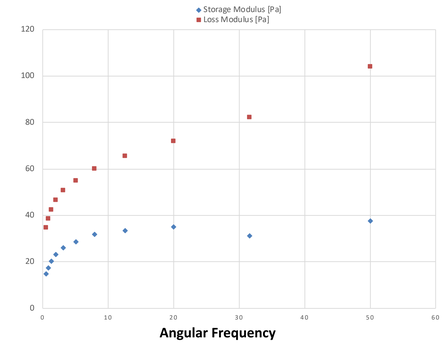

Figure 4: Storage (G') and loss (G'') moduli of the bioink containing fibrin, alginate, and genipin crosslinked with crosslinker containing calcium chloride, chitosan, and thrombin without cells were measured using Anton-PAAR Rheometer

CONCLUSION

Cell seeding density plays an important role in morphogenesis and differentiation of neural progenitors. Our results indicate that crosslinked bioink has good mechanical properties, and biocompatibility which are important factor for bioprinted tissues.

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[2]*https://www.aspectbiosystems.com/product-categories/rx1-bioprinter*

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