A comparative study on the suitability of bioinks for 3D bioprinted head and neck tumour model

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INTRODUCTION

The development of preclinical models for head and neck squamous cell carcinoma (HNSCC), an invasive and metastatic tumour, becomes essential for effective tailoring of novel therapies to improve the overall clinical outcome [1]. Owing to the substantial advancements in science and technology, 3D bioprinting technology, has gained the limelight in regenerative medicine and tissue-engineering fields due to its promising capability to mimic the heterogeneity of the native tumour niche [2]. In this study, we focused on engineering 3D bioprinted model for HNSCC, using extrusion-based bioprinting. Our study goal is to determine the optimal bioprinting parameters; comparing different natural & marine- derived nanocellulose (NC) - based bioinks to the most commonly used semi- synthetic gelatin methacryloyl (GelMA) - based hydrogel, in specific to fabricating 3D *in-vitro* bioprinted HNSCC model.

MATERIALS AND METHODS

After designing a 3D cylindrical structure, we conducted our initial set of trial experiments optimising their respective critical parameters. Once the bioprinting parameters were optimized, we printed the designed structure with HNSCC cell-laden (UM-SCC- 14C, 11B & 22B) bioinks in different tunicate derived NC bioinks (Bioink 1-TEMPO-mediated oxidised NC; Bioink 2–Carboxymethylated NC) comparing to gelatin-based bioink (Bioink 3- gelatin methacrylate in alginate GelMAA). Further, we investigated the varied bioink biocompatibilities using chemiluminescence- based 3D viability assay kit.

RESULTS AND DISCUSSION

We cultured our 3D bioprinted HNSCC constructs for 21 days characterizing them for their viability over the culture period. We observed UM-SCC-22B cell-laden bioconstructs in bioink 2 showed relatively higher viability than cells in bioink 1. Furthermore, the cell survival of HNSCC cells in bioink 2 were comparable to the bioink 3. In addition, the viability of UM-SCC-22B cells in NC bioink were significantly higher than UM-SCC-14C & 11B cells in the 3D bioprinted constructs. Similarly, when the crosslinker concentration was altered from 50mM to 20mM $CaCl_2$, we observed an increase in overall viability.

CONCLUSIONS

The witnessed differences in bioink biocompatibilities could be prominently due to the differences in bio-chemical & mechanical properties. Nevertheless, bioink 2 supports cell proliferation & matrix modification owing to its carboxymethyl backbone whereas bioink 1's carboxyl backbone limits equivalent cell behaviour due to the presence of heavy functional group. In addition, NC fiber distribution differences might additionally affect viability. The observed enhancement in viability with lowered crosslinker concentration indicates the reduced Ca^{2+} ions favoured cell survival [3]. Moreover, bioink 2 behaves similar to the standardly used GelMAA. Our current findings lays forward the pioneer steps aimed at the development of a 3D-bioprinted HNSCC preclinical model with a highly biocompatible, mechanically stable and naturally- derived nanocellulose hydrogel.

CONFLICT OF INTEREST

Our research group has a well-established collaboration with Ocean Tunicell AS, Norway (which manufacturers the hydrogel used in this project) providing significant involvement in the project progress.

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