Development and optimisation of highly cellular, embedded bioprinting strategies for the 3D bioprinting of functional cartilage grafts

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INTRODUCTION

Tissue engineering (TE) strategies aim to regenerate tissues which have lost significant functionality due to physical damage or disease. Current approaches fail to recapitulate the highly organised structure and extracellular matrix (ECM) composition of complex tissues such as articular cartilage, motivating the exploration of novel TE strategies. 3D Bioprinting enables the precise deposition of bioinks in a spatially organised, three-dimensional manner, potentially enabling the engineering of complex tissues and organs. Embedded bioprinting is a recent advancement within the field of 3D bioprinting which enables the production of complex, free-form constructs using soft hydrogels, or cell-only bioinks. The objectives of this work were to develop and optimise highly cellular, embedded bioprinting strategies for 3D bioprinting of functional cartilage grafts. Specifically, work has been undertaken to develop highly cellular alginate-based bioinks, and to develop and optimise two embedded printing strategies, an amended freeform embedded suspended hydrogel (FRESH)[1] strategy, and an oxidised-methacrylated microgel (OMA)[2] (cell-only) strategy, as well at the print process to produce spatially defined, functional tissues with an organised collagen microarchitecture.

MATERIALS AND METHODS

gBMMSCs ($60x10^6$ cells/ml) were encapsulated in an oxidised alginate (4% oxidation) bioink[3]. A non-oxidised, acellular alginate bioink was also prepared for the printing of support structures/boundary walls. Prior to use, the bioinks were assessed rheologically. The cell laden bioink was first deposited in agarose moulds and cultured in chondrogenic medium for 6 weeks, to assess the capability of the oxidised alginate bioink toward supporting chondrogenesis, confirmed via biochemical and histological analysis. Both of the embedded printing strategies (FRESH, OMA) were characterised using rheology. Acellular printing was undertaken to assess the capabilities of both strategies regarding resolution and fidelity of printed constructs. Bioprinting of various constructs was then undertaken, with a non-oxidised alginate support structure and a core of the cell laden oxidised alginate bioink. The constructs were maintained in chondrogenic culture for 6 weeks, residual alginate bioink removed, and then assessed via live/dead staining, biochemically, histologically and using polarised light microscopy.

RESULTS AND DISCUSSION

The cell laden bioink casted into agarose moulds supported robust chondrogenesis, with high levels of sulphated-glycosaminoglycans (sGAGs) (60 µg), and collagen/DNA production (60 µg/µg), further confirmed through histological evaluation. Both embedded printing strategies were able to support the high resolution bioprinting of both the oxidised alginate (100 µm) and non-oxidised alginate (150 µm) bioinks of constructs with good fidelity and viability. The print process was optimised toward the accurate bioprinting of multiple-material isotropic cubed constructs. Following a period of two weeks, the oxidised alginate bioink had fully degraded. The bioprinted cubes maintained good fidelity throughout the culture period, and the gBMMSCs contracted sufficiently into microtissues. Homogeneous chondrogenic extracellular matrix was produced throughout the culture period. Biochemical and histological analysis presented with robust chondrogenesis, demonstrated via chondrogenic ECM secretion (sGAGs and collagen), further confirmed via homogenous histological staining of sGAGs and collagen.

CONCLUSIONS

Initial work has been undertaken in the development and optimisation of highly cellular, embedded bioprinting strategies toward the 3d bioprinting of functional cartilage grafts, including bioink development and characterisation, embedded bioprinting material development and characterisation, optimisation of the multi-material, sterile embedded printing process and chondrogenic characterisation of the potential of mentioned cartilage grafts. Future work will continue to endeavour to engineer optimized physiologically relevant grafts with a highly organised spatial composition and mechanical properties akin to the native tissue, utilising printed boundary conditions to direct the organisation of developing tissue.

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