Suspended additive manufacturing of complex wounds for precision therapy testing.

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INTRODUCTION

Complex wounds are a significant healthcare burden, and their treatment is challenging because of multiple treatment and clinical episodes, social isolation, prolonged hospitalisation periods, increased morbidity, and even amputation. To address this problem, management spans health optimisation, dressings, and surgery. However, the efficiency of such therapies is still limited by the lack of physiologically relevant, reproducible, and personalised wound models often leading to failed clinical trials and increased rejection chances. Although several models have emerged to target these issues, the lack of complexity in their biochemical mechanisms, and their poor knowledge of the wound-healing process highlights the urgency of developing a proper human complex wound model. Here we challenge the current perspective and propose the integration of advanced photopolymers with primary dermal cells and 3D bioprinting technology to generate a multi-layered model capable of mimicking the structure and composition of native human skin. Different hyaluronan-based inks with tuneable physical properties are formulated and blended with collagen to create IPN's capable of supporting the encapsulation and function of fibroblasts and keratinocytes. Preliminary results confirm high levels of cell viability and phenotype retention post-printing, suggesting this model can be used to interrogate complex cell-cell and cell-matrix interactions during wound healing.

MATERIALS AND METHODS

Visible light crosslinkable hyaluronic-based hydrogels were developed by the group of Dr. Ana Fonseca (University of Coimbra) and further modified with collagen type 1 (0.5% w/v) to obtain an ink with suitable physicochemical properties for 3D bioprinting of skin models. Photorheology and oscillatory rheology measurements including flow and amplitude sweeps were performed to evaluate both curing kinetics and the printability of developed polymeric inks. Primary human dermal fibroblast and primary human epidermal keratinocytes were embedded in the hydrogel at a density of $3x10^6$ cells/mL and $2x10^6$ cells/mL, respectively to obtain bioinks. The latter were loaded into sterilised cartridges and printed in suspension using a multimaterial bioprinter (BioX6, CELLINK). Cell viability and gene expression were evaluated at days 4, 7, and 14 post-printing using LIVE/DEAD and RT-qPCR respectively.

RESULTS AND DISCUSSION

Photorheology measurements show a fast gelation process of developed bioinks with full crosslinking being achieved just after 12 seconds of irradiation with visible blue light. Flow sweep measurements confirm that all formulations display a shear thinning behaviour typical of pseudoplastic materials i.e. decreasing viscosity with increasing deformation. Amplitude sweep tests reveal that all formulations exhibit a typical viscoelastic behaviour with constant and independent storage (G') and loss (G") moduli response to increasing strain. Live/Dead results show high levels of cell viability in all printed constructs at all time points with little or no variation compared to manual encapsulated cells. Finally, the PCR results showed that the genes studied were akin to the control in 2D which means that the hydrogel and the application of the blue light did not affect either the genetic profile of the cells or their activity.

CONCLUSIONS

Here we have proposed the integration of new hyaluronan-based photopolymers with extrusion and visible light-assisted bioprinting to create 3D multi-layered models of human skin. Preliminary data suggest that the developed polymeric inks can be fully crosslinked in less than 12 seconds without inducing any significant cytotoxicity and are indeed supportive of cell phenotype expression and maintenance in 3D. Further work will be carried out to validate the potential of developed bioinks in dermal applications whilst increasing the complexity of the model with the addition of other skin layers and resident cells.

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