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Full Length Article

Simulating image-guided *in situ* bioprinting of a skin graft onto a phantom burn wound bed

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ABSTRACT

Deep skin thermal wounds require skin excision and engraftment. Clinical treatment for deep skin wounds is the use of autologous split-thickness skin grafts. However, timely coverage of large-area burn wounds remains a significant challenge. Engineered skin tissue constructs aim to overcome the limitations of traditional clinical intervention. In this study, an *in situ* bioprinting-based methodological workflow is advanced to directly fabricate a custom engineered skin graft onto a skin burn phantom. To illustrate this modular approach, a burn phantom is first created by mold casting gelatin-alginate hydrogel material to simulate a burn wound bed with arbitrary 2D shape and uniform depth. The cast hydrogel phantom is then placed on the printer platform to host the to-be-printed skin graft. Next, a color image-based module is proposed to extract the contour of the burn wound. This is followed by implementing a contour calibration process based on fiducial markers to yield the real dimension and pose of the burn phantom. A new directed toolpath generation algorithm is detailed to generate a burn-specific toolpath for the microextrusion-based bioprinting process. Based on this method, the bioprinted cell-laden gelatin-alginate hydrogel filaments are precisely arranged in a meshed pattern that is bound by the burn phantom contour. Internal geometries defined by the filament and pore dimensional characteristics of the printed construct design can be controlled to promote cell viability, proliferation, and nutrient delivery. Printed cell-laden multi-layered constructs are evaluated for single filament and pore dimensional precision, alignment of filaments between layers, and positional accuracy of the filaments within the extracted contour. Finally, a 24-hour time course incubation study reveals that the printed constructs preserve their structural properties while cells proliferate and maintain their spatial positioning.

1. Introduction

Globally, an estimated 265 000 deaths per year are caused by burns [1]. In addition to functional skin loss, susceptibility to wound infection leads to multi-organ failure. As such, the appropriately timed selection and implementation of the clinical treatment modality are fundamental to reducing burn mortality rates [2–5]. Thousands of individuals require skin transplants to treat the large number of clinical cases of deep burn wounds [6] In practice, the prevailing tissue used in skin transplantation surgery is the autograft where a healthy piece of one's own healthy skin tissue is harvested from an ectopic site to cover the burn wound area [7]. The surgery is performed by generating an epidermal sheet of cultured keratinocytes, and followed by transplantation of the epidermal sheet in conjunction with a dermal substrate onto the injury [8]. This includes split-thickness skin autografts (STG) for small areas [9]. However, for severely injured patients with multiple depth wounds and indeterminate burns covering a large areal surface of the body, harvesting of autografts is dimensionally limited to areas unaffected by injury [10]. Also, a patient with large wounds must receive immediate wound coverage to promote wound healing. Current use of STGs for full-thickness burns frequently result in scarring and the absence of dermal tissue support with contractures [11]. Another treatment modality is cell therapy, which entails spraying therapeutic cells to deliver the requisite cells to the wound area. The cell therapy provides a clinically acceptable functional outcome. However, this uncontrollable cell delivery method lacks the spatial precision needed to reliably deliver cells to the specific wound sites. Furthermore, these existing approaches lack the ability to produce large and complex constructs with complex geometries and composition towards functional, aesthetic skin mimics. To overcome the limitations of autografting and pure cell therapy, engineered skin tissues using cell-laden biomaterials are under investigation to efficiently resurface thermal wounds [12].

As an alternative to conventional grafting, emerging advanced manufacturing technologies enable engineered analogs of skin tissue to be produced. A prevailing bioprinting process is the microextrusion-based system which has specifically been applied to create biologically

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active patient-specific skin tissue with custom geometries [13–16]. Although such methods enable the layered fabrication of complex 3D biopolymer structures, for these manufactured systems to have clinical utility, cells must exhibit biological functionality within the printed structure. Specifically, by prescribing 3D microenvironments which mimic key features of a physiological extracellular matrix, cells will be coaxed to express *in vivo* functionality. Based on this design principle, the microenvironmental control in bioprinting captures the structural and functional features of native skin [17–19].

Developing a custom graft is contingent on the interplay between the material or bio-ink selection and the spatial design of the graft. A useful class of biomaterials for bioprinting are hydrogels, water-laden polymers formed by crosslinking. These materials are physically and chemically mild for cytocompability, possess similar mechanical properties to living tissue, and facilitate nutrient and waste diffusion. Specifically, gelatin-alginate hydrogels have been widely adopted for their mechanical and controllable gelation properties [20,21]. However, key manufacturing challenges arising with bioprinted materials processing can be referred to the thermal sensitivity and high water composition of the gelatin-based bio-ink material is that the material consists primarily of water which is thermal sensitive. This makes it difficult to produce intricate features, such as pores, owing to the material susceptibility to undergo a subsidence phenomenon. Addressing this challenge would enable precise control of the key dimensional parameters, including printed filament diameter, inter-filament spacing and pore geometries critical for viable cell growth and function in the context of high structural fidelity of for engineered skin grafts.

To date, significant progress has been made towards engineering skin with functional attributes by fabricating multilayered structures and establishing strategies for tissue vascularization and innervation [22]. However, the particular scope of the current proposed methodology is *in situ* bioprinting of burn-specific grafts. Some work has previously been done to develop *in situ* printing methodologies and prototypes. For example, from the process innovation standpoint, the design of cell-laden biomaterial delivery systems by using inkjet microvalve printheads have been proposed to treat skin burns *in situ* [23]. To meet biological performance demands, the *in situ* printing of nano-hydroxyapatite material in a calvarial defect on mice by a laser-assisted bioprinting process has been reported [24]. In satisfying criteria for the mechanics of materials, an *in situ* double-light-source curing method has been reported for printing poly(ethylene glycol) diacrylate (PEDGA) hydrogel onto a defect 3D model [25]. Towards ease of use and portability, a handheld co-axial bioprinting technology has recently been demonstrated for *in situ* surgical cartilage repair accompanied by high biological performance [26]. Although each of the aforementioned techniques manage to highlight the various critical considerations along the *in situ* bioprinting process chain, gaps in key knowledge and procedural details of the workflow should be clearly defined and integrated to optimize the *in situ* bioprinting methodology. To enable *in situ* bioprinting, process multiparametric optimization is necessary to ensure printability of the prescribed bio-ink. Moreover, an optical sensor or imaging modality must preliminarily ascertain the structure of the skin wound site in order to accurately deposit the cell-laden bio-ink. In order to specify the workflow of the proposed *in situ* printing methodology, several steps are introduced. First, a gelatin-based tissue phantom is designed to simulate the burn wound with a complex 2D geometry and a prescribed depth (ranging from 2 to 5 mm). The rationale for assigning a concave 2D polygonal geometry to the burn phantom derives from the author's previously reported work in which the burn wound contour is determined based on hyperspectral image acquisition [27]. As previously reported, hyperspectral imaging represents a noninvasive imaging method with hemodynamic indicators to guide the surgical debridement or excision of deep burn tissue. Upon excision, the residual burn area will feature distinct edges amenable to image-based discrimination. In the proposed methodology herein, a generic color image is implemented to calibrate the contour for a burn area simulated by an arbitrary burn phantom design. First, a color segmentation algorithm is applied to extract the 2D contour of the prescribed burn area. In order to calibrate the accurate position of the phantom, a set of fiducial markers is then defined to identify the transformation matrix between the respective coordinate systems for the image space and printer platform. Then, the calibrated 2D contour is converted to a 2D continuous toolpath based on the proposed continuous direction-based toolpath. This is generated using an adaptive gap algorithm to guide the direct bioprinting of a burn-specific skin graft with a meshed internal structure and prescribed depth profile.

2. Materials and methods

2.1. Cell culture

Culture of human skin primary fibroblast cells (Coriell) is maintained in Dulbecco's Modified Eagle's medium (ATCC) and 15% fetal bovine serum (ATCC), with pen/strep antibiotics (ATCC) and incubation conditions of 37 °C at 5% CO_2. Subculture of cells with 0.25% trypsin (ATCC) and prepared for bioprinting experiments when cell monolayer coverage reaches approximately 90%.

2.2. Preparation of hydrogels

Hydrogel pre-polymer material concentrations of 20% w/v type A gelatin (Sigma) and sodium alginate (Sigma) are prepared by dissolution in Dulbecco's phosphate buffered saline (ATCC) with overnight magnetic stirring on a hot plate setting of 95 °C. Serial filtration (0.45 μm and 0.20 μm pore sizes) of the gelatin-alginate solution proceeds at prescribed volumetric ratios. The alginate hydrogel crosslinking solution is prepared with dissolution of 5% w/v calcium chloride ($CaCl_2$) in deionized water.

2.3. Formulation of bio-ink

The acellular pre-polymer bioprint material with formulated with equal parts of gelatin (20% w/v) and alginate (2% w/v). The fibroblast cells are centrifuged and suspended in a 0.5 ml solution of DPBS. To this cell suspension, 0.5 ml of gelatin (20% w/v)-alginate (2% w/v) solution is additively mixed at 37 °C. The resultant bio-ink material formulation contains fibroblast cells suspended in gelatin (10% w/v)-alginate (1% w/v) solution. For the *in situ* bioprinting experiments, the cell seeding density is prescribed at 12×10^6 cells/ml.

2.4. 3D bioprinter system configuration

The bioprinted tissue fabrication platform is enabled by modifying the Fab@home (2^{nd} generation) 3D material extruder with a syringe-based print head mount. The three-dimensional stage translation for precise spatial deposition of material is controlled using Motion Basic software (Jeffrey Kerr, LLC). An integrated heating unit is implemented for process control of the material temperature parameters. A digital web camera is positioned to enable image acquisition of the printing platform. The bio-ink material is loaded into the 3 ml syringe-based material reservoir as shown in Fig. 1. A 22 gauge needle tip (EFD, Inc.) is adapted to the syringe print head. The volumetric rate of the bio-ink is prescribed at 5 ml/hr based on the rotational speed of the motor along with a path speed of 10 mm/s.

2.5. Skin burn phantom model design

Clinically, for a skin graft candidate, burn wound tissue is removed in order to ascertain that a suitable wound bed is available as confirmed from visible bleeding capillary networks [28]. Prior to engraftment, the clinical excision process typically yields a modified burn wound structure with a characteristically uniform depth. In this study, a skin burn