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Patient-specific 3D scanned and 3D printed antimicrobial polycaprolactone wound dressings

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ABSTRACT

The increasing prevalence of wound infections caused by antibiotic resistant bacteria is an urgent challenge facing modern medicine. To address this issue the expedient use of antimicrobial metals such as zinc, copper and silver were incorporated into an FDA-approved polymer (polycaprolactone – PCL) to produce filaments for 3D printing. These metals have broad-spectrum antimicrobial properties, and moreover, copper and zinc can enhance the wound healing process. 3D scanning was used to construct 3D models of a nose and ear to provide the opportunity to customize shape and size of a wound dressing to an individual patient. Hot melt extrusion was used to extrude pellets obtained by vacuum-drying of solutions of PCL and the different metals in order to manufacture metal-homogeneously-loaded filaments. Wound dressings with different shapes were produced with the filaments containing different concentrations of metals. Release of the metals from the dressings was determined by inductively coupled plasma atomic emission spectroscopy. All the different metal dressings show fast release (up to 24 h) followed by slow release (up to 72 h). The antibacterial efficacy of the wound dressings was tested using a thermal activity monitor system, revealing that silver and copper wound dressings had the most potent bactericidal properties. This study shows that 3D scanning and 3D printing, which are becoming simpler and more affordable, have the potential to offer solutions to produce personalised wound dressings.

1. Introduction

The skin is the largest organ in the body, functioning as a sensory system, regulating both temperature and moisture transmission and acts as a physical barrier against the external environment. When a wound occurs, due to trauma or disease, the barrier becomes compromised. This can increase the susceptibility of the wound site to microbial infections originating from endogenous sources, such as surrounding skin and mucous membranes, or from exogenous sources, such as those introduced by injury or from the local environment (Landis, 2008). The introduced microorganism may overcome the host's defences and invade into deeper tissues, progressing to a more severe infection, thus causing further damage and delaying healing of the wound (Siddiqui and Bernstein, 2010).

A wound may require the application of an external dressing to temporarily compensate for the damaged barrier and to allow healing to initiate and progress. A wound dressing isolates the injury site from the external environment and provides an optimal environment for the wound to heal by promoting haemostasis and limiting tissue oedema through external compression (Zahedi et al., 2010). Wound dressings, traditionally used to protect the wound from contamination, can be used as platforms to deliver actives to wound sites. The use of solid wound dressings is preferred to the use of topical bioactive agents in the form of solutions, creams, and ointments in the case of exudative wounds for drug delivery to the wound as they provide better exudate management and prolonged residence at the wound site. These dressings are potentially useful in the treatment of local infections being beneficial to achieve increased local concentrations of antibiotics while avoiding systemic treatment, thus reducing patient exposure to an excess of drug beyond that required at the wound site (Boateng and Catanzano, 2015).

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Due to the alarming increase of multi-drug resistance bacteria worldwide, caused by the over-use and miss-use of antibiotics, the application of broad-spectrum antimicrobial agents such as metal ions is an attractive target. Having been used historically for their antimicrobial properties (Lemire et al., 2013; Tenaud et al., 2009), the use of inorganic antimicrobial metals in the fight against infections is of high importance due to the fact that they act on multiple bacterial pathways, which makes it difficult for the bacteria to develop resistance against them (Huh and Kwon, 2011). Silver is probably the most commonly used metal, but zinc and copper, two of the essential trace elements in the human body, are also known to play an integral part in the wound healing process.

Silver ions have been shown to bind to various bacterial cell membrane proteins to cause cell lysis, and can be transported into bacterial cells, where silver ions disrupt the cell wall to interfere with energy production, enzyme function, cell replication and ultimately cell death (Chopra, 2007; Fong and Wood, 2006; Jain et al., 2009). There remains a concern in relation to the toxicity of silver to humans, however, most frequent side effects including local skin irritation, discolouration or staining which are harmless and usually reversible (Cutting et al., 2007). Copper ions function by altering proteins and inhibiting their biological activity, membrane lipid peroxidation, and plasma membrane permeabilization (Borkow and Gabbay, 2005; Gabbay et al., 2006). Copper can improve the healing process as it plays a key role in the enhancement of angiogenesis, via induction of vascular endothelial growth factor (VEGF), up-regulating the activity of copper-dependent enzymes, cell proliferation and re-epithelisation (Liu et al., 2009). It is suggested that the mode of action of ZnO is due to the disruption of bacterial cell membranes, and zinc is involved in several transcription factors and enzyme systems, stimulates the proliferation of epidermal cells, and increases collagen synthesis. Topical zinc can improve the healing of wounds especially in patients with zinc deficiency (Lemire et al., 2013), which can be a result of hereditary causes (Lansdown et al., 2007).

Wound dressings are usually prepared from absorbent, cross-linked polymer networks. One potential polymer is polycaprolactone (PCL), a semi-crystalline polyester that is biodegradable and biocompatible. These properties have led to the approval of several PCL drug-delivery devices and implants by the FDA (Salgado et al., 2012). It has a slow rate of degradation *in-vivo* compared with other biodegradable polyesters, a property that can be exploited in the manufacture of controlled release formulations (Li et al., 2014). PCL has been widely investigated in wound and burn dressings (Boateng et al., 2008; Ng et al., 2007), tissue engineering (Kweon et al., 2003), scaffold manufacturing (Kamath et al., 2014) and drug targeting (Freiberg and Zhu, 2004).

Three-dimensional printing (3DP) is a recently developed technology with numerous possibilities for the manufacture of medical devices. 3DP is an additive manufacturing process that allows the fabrication of three dimensional solid objects of virtually any shape. Of the several types of 3D printing, fused deposition modelling (FDM) has been most widely used for medical devices as it is simple, cost effective and extrudes polymer strands (Goyanes et al., 2016a; Yu et al., 2008). The printer feedstock is a thermoplastic filament that is heated to its softening point and then extruded through a print-head (driven by an X – Y orientation system) layer by layer over a build plate. The build plate is then lowered to a predetermined height and the process is repeated until the 3D object has been constructed. FDM 3DP has been used in various fields, such as tissue engineering, scaffold manufacturing (Fielding et al., 2012), and to produce oral drug delivery formulations (Goyanes et al., 2014, 2015a,b, 2016b; Melocchi et al., 2015; Pietrzak et al., 2015). The 'instructions' for the 3D printer on how to build the object comes from the printer's software that slices the digital source file into layers that form the instructions for the 3D printer. This digital file can be created using computer-aided design software, to construct a new 3D object, or with the use of 3D scanning, to copy an existing object. 3D scanning is a non-contact, non-destructive technology that digitally captures the shape of physical objects with a 3D scanner using laser light that collects distance information from surfaces. This information is then used to create 'point clouds' of data from the surface of the object. Hence, 3D laser scanning is a way to capture a physical object's exact size and shape to construct a 3D model (Koch, 2012). The proof of concept of combining 3D printing and 3D scanning for the manufacture of antiacne masks/patches has been previously reported (Goyanes et al., 2016a), whereas the use of FDM printing showed high drug degradation due to the heating process while printing.

The combination of 3D printing and 3D scanning could possibly revolutionise patient care by allowing custom-manufacture of devices for individual patients and it is the exploration of this concept, applied specifically to wound dressings, that is the focus of this work. Hot melt extrusion was used to incorporate metal ions into a PCL filament and the 3D printer was used to fabricate dressings against scanned templates of a target wound. The antimicrobial efficacy of the dressings was also assessed using an *in-vitro* assay.

2. Materials and methods

2.1. Materials

PCL pellets ($(C_6H_{10}O_2)n$, Mw ~ 80,000) and silver nitrate ($AgNO_3$) were purchased from Sigma-Aldrich, UK. Copper sulphate (II) pentahydrate ($CuSO_4{\cdot}5H_2O$) was purchased from VWR chemicals, Belgium. Zinc oxide (ZnO) was purchased from Alfa Aesar, USA. The test organism *Staphylococcus aureus* (NCIMB 9518) was purchased from Fisher Scientific, UK. Nutrient broth (CM0001) was purchased from Thermo Scientific, UK.

2.2. Methods

2.2.1. Preparation of metal loaded filaments

- Silver-loaded filament (10% loading w/w):

$AgNO_3$ (3 g) was dissolved in 10 mL of deionized water using a magnetic stirrer. Tetrahydrofuran (THF, 200 mL) was added to the silver solution under stirring. Finally, 27 g of PCL pellets was then added to the solution and the mixture was stirred at 40 °C until complete dissolution of PCL. The solvents were removed with a rotary evaporator under reduced pressure at 40 °C for 2 h followed by high-vacuum drying for 1 h. The dried material ($AgNO_3$ homogeneously distributed in the PCL) was chopped into pellets and extruded with Filabot filament hot-melt extruder (Filabot Inc, USA) with a single screw and a 1.75 mm nozzle head. The extrusion temperature was 80 °C.

- Copper-loaded filament (10 and 25% loading w/w):

$CuSO_4{\cdot}5H_2O$ (3 g or 7.5 g for 10% or 25% loading respectively) was dissolved in 100 mL methanol using a magnetic stirrer. PCL pellets (27 g or 22.5 g for 10% or 25% copper loading respectively) was then added to the copper solution, followed by 100 mL dichloromethane (DCM) and the mixture was stirred at 40 °C until complete dissolution of PCL. A rotary evaporator (under reduced pressure) was used to evaporate the solvents at 40 °C for 3 h followed by high-vacuum drying for 1 h. The dried material ($CuSO_4$ homogeneously distributed in the PCL) was chopped into pellets and extruded with Filabot filament hot-melt extruder (Filabot Inc,