Biomaterials 32 (2011) 279–287

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Polycaprolactone-based fused deposition modeled mesh for delivery of antibacterial agents to infected wounds

Erin Yiling Teo [a], Shin-Yeu Ong [b], Mark Seow Khoon Chong [c], Zhiyong Zhang [a], Jia Lu [b], Shabbir Moochhala [b], Bow Ho [d], Swee-Hin Teoh [a,e,*]

[a] Centre for Biomedical Materials Applications and Technology (BIOMAT), Department of Mechanical Engineering, Faculty of Engineering, National University of Singapore, Singapore
[b] Combat Care Lab, Defence Medical & Environmental Research Institute, DSO National Laboratories, Singapore
[c] Experimental Fetal Medicine Group, Department of Obstetrics & Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[d] Department of Microbiology, National University of Singapore, Singapore
[e] National University of Singapore Tissue Engineering Programme (NUSTEP), National University of Singapore, Singapore

ARTICLE INFO

Article history:
Received 10 August 2010
Accepted 27 August 2010
Available online 26 September 2010

Keywords:
Polycaprolactone–tricalcium phosphate
Fused deposition modeling
Antibiotic delivery system
Infection control

ABSTRACT

Infections represent a significant source of site morbidity following tissue trauma. Scarring and tissue adhesion remain the challenging issues yet to be solved. Prolonged inflammation and morphology of the re-epithelisated layer are important considerations. We hypothesized that the solution lies not only in the biochemistry of biomaterial but also the micro-architecture of the scaffold used as the matrix for wound healing. Targeted delivery of antibiotics may provide an efficacious means of infection control through adequate release. Here, we study the use of 3-dimensional polycaprolactone–tricalcium phosphate (PCL–TCP) mesh for the delivery of gentamicin sulphate (GS) fabricated using a solvent-free method. PCL–TCP meshes incorporated with varying loads of GS were evaluated *in vitro* for elution profile, antimicrobial efficacy and cytotoxicity. Results showed that PCL–TCP meshes incorporated with 15wt% GS (PT15) efficiently eliminate bacteria within 2 h and demonstrate low cytotoxicity. Subsequently, PT15 meshes were evaluated using an infected full thickness wound mice model, and observed to eliminate bacteria in the wounds effectively. Additionally, mice from the PT15 treatment group (TG) showed no observable signs of overall infection through neutrophil count by day 7 and displayed efficient wound healing (94.2% wound area reduction) by day 14. Histology also showed significantly faster healing in TG through neo-collagen deposition and wound re-epithelisation. The meshes from TG were also observed to be expelled from wounds while gauze fibers from CG were integrated into wounds during healing.

1. Introduction

Infections following tissue trauma and surgery are a significant cause of site morbidity and can often lead to implant failure [1]. It has been reported that 22% of revision operations for orthopedic implant failure were due to implant infection [2], which ultimately increases the healthcare cost [2–4]. Alternatively, infections may be managed by the systemic administration of antibiotics either prophylactically or upon onset of an infection. However, systemic antibiotic therapy is often associated with the development of antibiotic resistant bacterial strains, as well as higher risks of systemic poisoning due to larger amount of drugs being required to achieve efficacious concentrations at the infection site [5–7]. Thus, it has been suggested that directed delivery of antibiotics to the infection site at a minimal inhibitory concentration has been shown to be a safer and more effective approach [1,8,9]. For example, directed antibiotic delivery is used to treat osteomyelitis, a prolonged inflammation of the bone that brings about destruction of bone tissues and vascular channels caused by pathogenic microorganisms [10,11]. Alternatively, direct delivery of antibiotics is also beneficial for other purposes such as wound dressings, where lower concentrations of antibiotics need to be given as compared to systemic administration, which usually involves excessive doses to achieve substantial local effects. This approach may also be useful in other infection control applications such as wound dressings, tissue engineering scaffolds and surgical patch.

* Corresponding author. Centre for Biomedical Materials Applications and Technology (BIOMAT), Department of Mechanical Engineering, Faculty of Engineering, National University of Singapore, Singapore
E-mail address: mpetsh@nus.edu.sg (S.-H. Teoh).

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doi:10.1016/j.biomaterials.2010.08.089

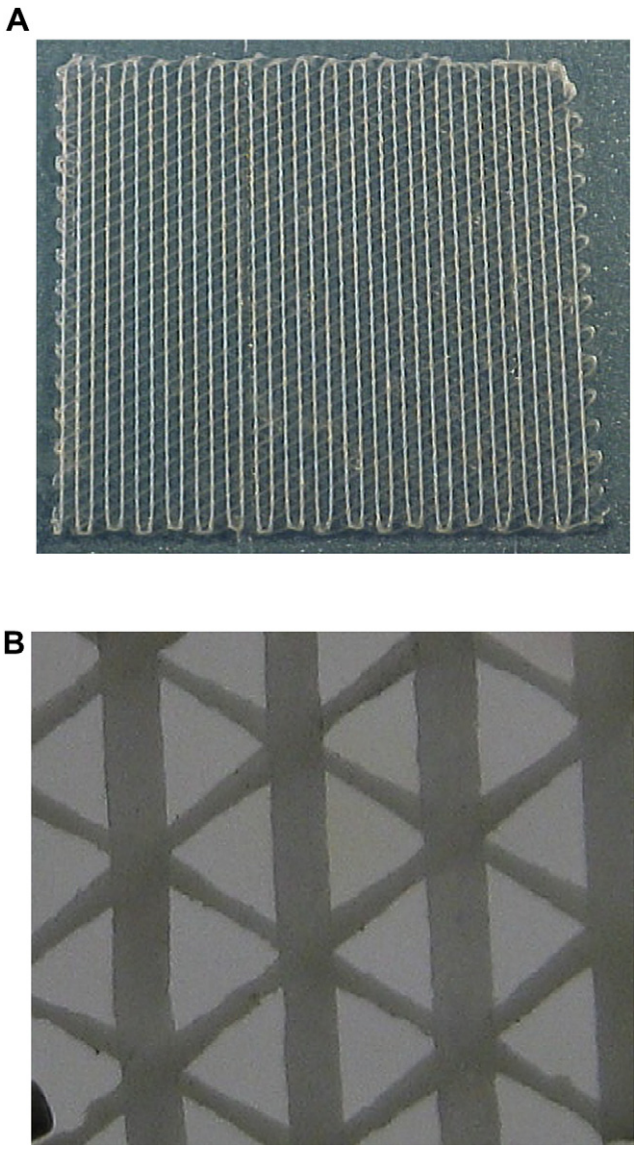

Fig. 1. A) PCL–TCP mesh incorporated with GS. (B) Enlarged image of the drug eluting PCL–TCP mesh.

Polymeric carriers have been studied extensively for the purpose of direct delivery of antibiotics with a wide range of applications ranging from tissue engineering scaffolds to topical wound dressings. The usage of biodegradable polymers as drug delivery platforms were regarded to be desirable as the system can be degraded and removed from the body naturally and without any adverse effects [12]. Polycaprolactone (PCL) is one such biodegradable polymer employed in a wide range of biomedical applications, which includes the Capronor implantable drug delivery system for sustained administration of hormonal contraceptives [13]. The high permeability of PCL resulting from its rubbery characteristics is often also used for delivery of low molecular weight drugs such as steroids and vaccines [14,15]. Our group had previously demonstrated the use of PCL, incorporated with 20wt% TCP as a delivery system for platelet-rich plasma and bone morphogenic protein-2 *in vitro* [16,17]. However, its delivery of low molecular weight hydrophilic antibiotics has not been explored.

Gentamicin sulphate (GS), a widely used aminoglycoside with antibacterial activity against both aerobic gram-positive and gram-negative bacteria, was chosen as the model drug for incorporation. Topical delivery via drug carriers may be used to overcome the limited bioavailablity of GS associated with oral or transdermal routes of administration due to its poor intestinal and dermal permeability [18]. Moreover, the general concern about risk of over toxicity and systemic toxicity can also be avoided when low amount incorporated can be released locally, targeting only the infection site [19]. Furthermore, GS is also heat-stable, and thus compatible with a range of fabrication and incorporation methods, including fused deposition.

For this study, PCL-20%TCP mesh incorporated with various percentages of gentamicin sulphate was fabricated and evaluated *in vitro* for its efficacy against gram-positive and gram-negative bacteria, elution efficiency and cytotoxicity using the dermal fibroblast cells. The appropriate GS incorporation concentration was then identified and used for further testing *in vivo* using a mouse full thickness infected wound model.

2. Materials and methods

All reagents were obtained from Sigma–Aldrich, Singapore unless otherwise stated.

2.1. Fabrication of antimicrobial incorporated mesh

PCL–TCP was blended with 5wt%, 15wt% and 25wt% of gentamicin sulphate (PT5, PT15, PT25). The physical blend was then fabricated into a 3-dimensional mesh with honeycomb-like pattern, interconnected pores and controllable porosity using the fused deposition modeling method. 50 × 50 × 1 mm matrices of 85% porosity and laydown pattern of 0°/60°/120° were obtained (Fig. 1). The PT5, PT15 and PT 25 meshes were then cut into discs of 6 mm in diameter in an aseptic manner for *in vitro* experiments.

2.2. In vitro analysis

2.2.1. Elution profile

GS elution was assayed using a protocol adapted from Aviv et al. [20]. Samples were immersed in 1.5 ml phosphate buffer saline (PBS) containing 0.05%w/v of sodium azide at 37 °C. The meshes were then removed and added into new vials of similar solution ($n = 5$) at the various timepoints of 0, 0.5, 2, 3.5, 5, 6, 24, 48, 72, 96, 120 and 168 h. The aliquots at various timepoints were frozen down to −80 °C for storage before analysis.

For spectrophotometry analysis, an equal amount of o-phthaldiadehyde reagent (2.5 g of o-phthaldiadehyde, 62.5 ml of methanol and 3 ml of 2-hydroxy-ehtyl-mercaptan and 560 ml of 0.04 M sodium borate in distilled water) and isopropanol were added to the aliquots and incubated in the dark for 45 min at room temperature. Absorbance at 332 nm was measured with blank o-phthaldiadehyde and isopropanol as control. All readings were done in duplicates and compared against a calibration curve generated from GS solutions of known concentrations.

2.2.2. Antimicrobial efficacy

Time kill assays adapted from previous studies [21,22] were conducted as follows. *Staphylococcus aureus* (ATCC25923) or *Pseudomonas aeruginosa* (ATCC27853) was prepared from tryptic soy broth (TSB) (Oxoid Ltd, Basingstoke, Hampshire, England) and concentration adjusted to 1×10^6 CFU/ml, based on 0.5 McFarland standards. Samples were then added to the bacterial broth culture, with untreated vials as growth control ($n = 3$). The vials were then incubated at 35 °C with constant agitation. 50 μl of bacterial broth was aliquoted from each vial at 0, 30 min, 2, 4, 24, 48 h, and plated after serial dilution to enumerate the number of surviving colonies following incubation for 24 h at 37 °C. Counts were used to establish the number of CFU per ml of TSB. Reinoculations of the bacterial broth culture of 1×10^6 CFU/ml were carried out at 24 h and 48 h.

2.2.3. Cytotoxicity assay

Cytotoxicity of the PT0, PT5, PT15, PT25 was evaluated according to ISO 10993 guidelines and with adaptation from Marques et al. and Burd et al. [23,24]. Briefly, 8×10^4 human dermal fibroblast cells cultured in antibiotic-free high glucose DMEM with L-glutamine and sodium pyruvate (Gibco), supplemented with 10% Fetal Bovine Serum (DMEM-FBS) were seeded to each well of a 12-well plate. The cells were allowed to adhere for 24 h before the aseptically prepared samples were added.

After 24, 48 and 72 h, WST-1 Cell Proliferation Reagent (Roche Molecular Biochemicals) was used to study cell viability according to manufacturer's instructions. Cell viability results for the different sample groups relative to the control group (PT0, cells in contact with PCL-20%TCP mesh without GS loaded) were obtained at each timepoint.