A novel sucrose porogen-based solid freeform fabrication system for bone scaffold manufacturing

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Abstract
Purpose – Musculoskeletal conditions are a major health concern in the USA because of a large aging population and increased occurrence of sport-related injuries. Bone tissue engineering may offer a less painful alternative to traditional bone grafts with lower risk of infection. The purpose of this paper is to present a novel porogen-based fabrication system for tissue engineering scaffolds using sucrose (C\textsubscript{12}H\textsubscript{22}O\textsubscript{11}) as the porogen building material.

Design/methodology/approach – A new solid freeform fabrication system has been developed and tested, which uses pressurized extrusion to print highly biocompatible and water soluble sucrose bone scaffold porogens (or negatives). Polycaprolactone (PCL) scaffolds are manufactured by injecting molten polymer into the porogens, and the porogens are subsequently dissolved with water. The resultant scaffolds demonstrate the defined porous structure designed into the sucrose porogen manufacturing computer-aided design model.

Findings – To optimize the porogen manufacturing process, the viscosity of sucrose mixtures is measured. Design of experiments is used to plan and analyze the relationships between the porogen characteristics and the process parameters. Reservoir pressure and print head speed are identified as the dominant factors affecting sucrose flow rate and porogen strut diameter, respectively. The biocompatibility of the new system is assessed by in vitro cell culture testing. Endothelial hybridoma cells (EAhy 926) and osteoblasts (7F2) seeded on the fabricated PCL scaffolds adhered to the scaffold and proliferated over four to six days. Epifluorescence and scanning electron microscopy images reveal cell spreading and multiple layers of cells on the scaffold surface. The results demonstrated the potential of the structured sucrose porogen-based fabrication method in manufacturing bone tissue scaffolds.

Originality/value – This paper describes the first time use of biomaterials—sucrose to make scaffold porogens and how an injection molded biopolymer scaffold can then be received.

Keywords Biotechnology, Bones, Injuries, Musculoskeletal system

Paper type Research paper

1. Introduction
More than 500,000 bone-grafting procedures are performed annually in the USA (Cutter and Mehrara, 2006). Autograft bone has been used for decades to supplement host repair and is still considered the gold standard for bone healing, but the amount of autograft is limited, and morbidity related to autograft harvesting can be considerable (Silber et al., 2003).

These shortcomings have prompted the development of bone tissue engineering. In tissue engineering, new tissues are created from cultured cells and biomaterials. A major goal of tissue engineering is to synthesize or regenerate tissues and organs. To achieve this goal, exploration and development of new biocompatible materials and manufacturing processes have been explored and developed to create synthetic bone grafts.

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A number of fabrication technologies have been studied to process biodegradable and bioresorbable materials into a 3D scaffold with high porosity and surface area. These technologies include porogen leaching, fiber bonding, gas foaming, and gel casting. Other scaffold fabrication methods include solution casting, emulsion freeze-drying, and textile (Gadzag et al., 1995; Chapekar, 2000; Mikos et al., 1993; Harris et al., 1998; Coombes and Heckman, 1992; Singhal et al., 1996; Mooney et al., 1996). These processes involve the use of organic solvents, high temperature melting or sintering and compression molding. Using the above-mentioned techniques is often difficult to produce functional structures with defined morphology which are important for the optimal tissue regeneration. Furthermore, most of the scaffolds for orthopedic applications made by these processes have relatively low mechanical strength, which may lead to implant failure and stress overloading. To overcome some of these problems, a porogen-based fabrication technology was proposed to fabricate porous scaffolds, and this porogen-based forming technology was developed based on the layer-by-layer manufacturing technique of solid freeform fabrication (SFF). SFF has been used for manufacturing scaffolds for tissue engineering for many years (Lange and Bhavnani, 1994; Sodian et al., 2002; Porter et al., 2001; Hutmacher, 2000; Park et al., 1998; Chu et al., 2001). These techniques are mainly limited by the fact that such SFF machines (for example, FDM, Solidscape, and all inkjet type 3D printing machines) must be adapted to the fluid mechanical properties of each biomaterial under consideration. The machine parameters must match the physical properties of the building material viscosity and surface tension. These properties vary significantly between different biomaterials and with temperature, making the use of a single machine for fabrication with multiple biomaterials difficult. The porogen-based fabrication system gives us the ability to use a single building material and the flexibility to use any compatible biomaterial or composite that can be injection molded (Mondrinos et al., 2006; Lu et al., 2005). From this research it has been found that the porogen method can reach higher resolution than that of direct build method (Lu, 2008).

The complete bone and tissue manufacturing system and its working principles are shown in Figure 1 with the following five specific steps:

1. Based on the multi-planar images obtained from computed tomography (CT) or magnetic resonance imaging (MRI), the 3D model of bone tissue outer shape/shell can be reconstructed first, then the internal structure of the bone extracellular matrix (ECM) scaffold will be built using bone structure bionic modeling. In this way, the 3D bone model with precise shape and similar internal structure as bone ECM will be digitally generated as a computer aided design model.

2. Based on the bone structure computer-aided design (CAD) model, a negative pattern of outer shell of bone and bone ECM can be fabricated by using SFF technology in stacking biocompatible sucrose.

3. A liquid-like gel of calcium phosphate (CaP) ceramics (CPC) and biopolymer composite is then injected into the negative sucrose skeleton to form the desired bone scaffold.

4. The negative sucrose skeleton is removed by immersing the assembly of the scaffold and skeleton into water.

5. The CPC biopolymer composite scaffold is then sintered to improve the mechanical integrity.

The resulting bone scaffold manufactured in this process takes the shape of the pores in the porogen, so the porogen is designed as the negative of the desired scaffold geometry.

In this work, we designed a novel porogen-based fabrication system for tissue engineering scaffolds using sucrose (C\textsubscript{12}H\textsubscript{22}O\textsubscript{11}) as the porogen building material. Since this is the first time sucrose has been introduced as a porogen material in SFF tissue engineering, the fabrication system design and control as well as the porogen material properties are presented in detail. The resulting polycaprolactone (PCL) scaffolds and their biocompatibility are also demonstrated.

2. Methodology

2.1 Fabrication system design

The SFF-based porogen fabrication system is shown in Figure 2. The control signal is sent from the host computer to move the nozzle in x-y directions based on the tool path. Air supply was connected with two regulators to provide compressed air for both the porogen material and the nozzle valve controller. The porogen material reservoir was wrapped with a band heater, which was controlled by the temperature controller. A custom-made copper needle tip was installed on the microvalve. This copper needle can be heated up to the same temperature as the microvalve. The heated and melted porogen material was then dispensed on the working stage which can move in the z-direction.

Build platform

This SFF-based porogen fabrication system used two DC motors (Pittman, Harleysville, PA, USA) to move the nozzle in x- and y-axis, and a stepper motor (Pittman) moves the stage in the z-axis. The motion in the x-y plane is driven with isochronous toothed belts. z-axis is driven with a lead screw.

Nozzle jet system (nozzle selection)

There are several constraints in the selection of the nozzle jet system. In order to simulate the channels in bone which contain the marrow, the nozzle must be able to generate small droplets. In addition, the reservoir, the nozzle, and the connecting tube must be able to withstand the temperature needed to melt the porogen material (80°C). There are two types of nozzles which can dispense small droplets, as we require: the air-driven needle valve and the piezoelectric nozzle. First, we used the piezoelectric jetting device with a 50 μm orifice from MicroFab Technologies, Inc. (Plano, TX, USA). The constraint of this nozzle is that it can only dispense a solution with viscosity < 40 cP. Preliminary tests established that a sucrose solution with such low viscosity cannot solidify quickly enough for layer by layer fabrication. An air pressure dispensing valve (EFD Inc., East Providence, RI, USA) can dispense very viscous materials up to 100,000 cP. Owing to the high viscosity of our porogen material, we first choose the HP 7X dispense system with highest pressure up to 560 psi from EFD. Because of the difficulty of controlling the HP 7X to get continuous flow with good quality, a 741MD-SS valve has been selected as needle valve in this study, which has an adjustable needle stroke with a unique calibration feature that allows us to maintain exact deposit size. This microvalve is ideal for automated assembly processes that require small dispensing tips ranging in size from 22 to 33 gauges. The dispense valve was wrapped in heat tape (OMEGA Engineering, Inc., Stamford, CT, USA) to maintain the temperature of the sucrose solution at about 90°C. A cement-on thermocouple (OMEGA) was placed between the heating tape and the valve to monitor...
the temperature. A commercial available polypropylene syringe barrel (EFD) as a reservoir was used to deliver the sucrose solution. The whole system was kept at 90°C to maintain the fluidity and good manufacturability of the sucrose solution. A heating element was used to keep the temperature of the porogen material constant. Fiberglass insulation was attached onto the sidewalls of the reservoir to prevent heat leakage. Cement-on thermocouples (OMEGA) installed on the self heating tube and reservoir can monitor the temperatures.

**Electronic system control**

The control system (Figure 3) is divided into three major parts: the temperature control, the motor control, and the EFD dispenser control. The sucrose temperature in the reservoir and nozzle system is maintained at 90°C by a temperature controller (CN8501TC, OMEGA) and a 10-A relay. The motion control system (Figure 4) uses a single motion controller for the x-y build platform and the Z-stage. The build stage holding the dispenser uses two DC motors (Pittman 9433J490 and Pittman 8322S003) to move the nozzle in the x- and y-directions. Both motors are driven through a motor amplifier (Pittman model 6409). The z-axis motion microcontroller controls a stepper motor (Superior Electric, Bristol, CT, USA) via a motor controller (Parker Hannifin – Compumotor Division, Rohnert Park, CA, USA).

**EFD dispenser control**

An automatic voltage regulation Microcontroller (AT90S8515, Atmel, San Jose, CA, USA) and ValveMate 7000 controller (EFD) are used to control the valve. By choosing the proper reservoir pressure, needle stroke and valve open time with the valve controller, the desired deposit size of the droplet can be achieved. Compressed air was utilized as extrusion force to extrude the sucrose porogen material under high pressure from the reservoir. Then the nozzle jet dispenses the sucrose onto the substrate. The nozzle jet is attached to the build stage, which can be moved in the x-y plane. When one layer is finished, the working table will move down a defined thickness and the machine will repeat the same process to build the next layer until the whole part is finished.

2.2 Porogen material study

Sucrose (C12H22O11), as one of the most abundant carbohydrates found in nature, is a major component of the food chain. It is highly soluble in water, and is also somewhat soluble in alcohol and other polar solvents. However, it is

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**Figure 1** (a) Diagram of the main processes in the bone scaffold and tissue manufacturing system and (b) a simplified illustration of bone scaffold fabrication processes: (i) sucrose structured porogen; (ii) Biopolymer composite injected into the skeleton cavity; (iii) resulting scaffold after the porogen was washed/leached out.
generally insoluble in non-polar solvents such as ether and chloroform. This selective solubility in different solvents provides a convenient processing manipulation, and will guarantee that a solution of composites polymer can be injected into the porous sucrose structure without destroying its structural integrity. The sucrose skeleton can be removed by using water leaching after the composite solidifies. At room temperature, sucrose is a monoclinic crystal. It melts at 185°C, and decomposes when heated above 200°C. Owing to its natural biocompatibility, sucrose has found broad applications in tissue engineering (Haines, 1981; Khan, 1984; Rubio et al., 1991). In this study, a sucrose composite was developed to build the negative skeleton (porogen).

Sucrose solution
Water and sucrose were heated together to 100°C until almost no water was left, and then honey and butter were added and manually stirred. The mixture was reheated to 85°C, and then alum was added. Using this mixture we could reduce the
melting temperature of the modified sucrose biomaterial from 185 to 80C.

The proportions of sucrose, water, alum, honey, and butter were determined by measuring the melt temperature and viscosity of the solution, and its solidification time. Viscosity was measured using a cone-plate viscometer (Rheometrics Fluid Spectrometer II, TA Instruments, New Castle, DE, USA) with Peltier temperature control. Viscosity was measured at 110, 120 and 135°C.

Structured scaffold and porogen design
In order to simulate the global pore structure present in bone tissue, a scaffold was designed with fully interconnected voids to enable the injection of scaffold material with a syringe. By using a syringe, pressure could be applied to the scaffold material to help overcome the frictional forces resisting material flow through the porogen. Three dimensional models of the scaffolds and corresponding porogens were created using Pro/Engineer. Pro/Engineer was also used to generate the stereolithography files required by the fabrication system.

2.3 Fabrication of scaffolds
PCL scaffolds were fabricated in following distinct steps. First, the PCL particles and porogen were placed in an oven (VWR 1410) at a temperature of 72°C. During heating, the PCL was occasionally agitated by hand and visually inspected for particles melting. After 1 h, the PCL and porogen were removed from the oven. The molten PCL was then quickly packed into a syringe and injected into the porogen. Excess PCL was removed with a spatula. The PCL was then allowed to cool to room temperature. Once at room temperature, the

filled porogen was placed in deionized (DI) water to separate the sucrose porogen material from the PCL. The DI water in the water bath was removed and replaced with new DI water for a minimum of three times. The PCL scaffold was then allowed to air-dry at room temperature.

2.4 Variable analysis and quality control
In order to improve the dimensional accuracy of the printed porogen and to evaluate significance of the input factors on flow rate of the printing sucrose in the nozzle and on strut size of printed porogen, design of experiments (DOEs) was used. DOEs is a structured and organized method for efficiently determining the relationship between a group of factors affecting a process and the output of that process, with minimal bias.

To measure the flow rate, the nozzle rack was positioned above an electronic balance with a Petri dish to collect the sucrose. The nozzle tip was approximately 1 cm above the Petri dish surface. All the experiments used the same nozzle tip with an inner diameter of 500 μm. An adjustable air supply was connected to the reservoir, which delivered the sucrose mixture to the microvalve for deposition. The mass flow rate was calculated from the change in mass over a 20 s interval. The mass flow rate measurements were recorded five times and the average was taken as the final value.

Variable analysis of flow rate
Eight-run factorial experiments were conducted on the flow rate of the micronozzle system and its nominal parameters are listed in Table I. Three factors representing most important settings for this machine, viz., pressure of the valve controller, temperature and pressure of the reservoir, were selected to be investigated in this sensitivity design because they were all closely related to the sucrose flow rate in the nozzle. The variations associated with the three selected factors are listed in Table II. A software package (DATAPLOT, NIST) was used to analyze the sensitivity of the flow rate to the different parameter settings (www.itl.nist.gov/div898/software/dataplot; Fong et al., 2006).

Variable analysis of strut size
The microvalve system deposits sucrose mixture onto substrate to form sucrose struts, which would then be oriented by the nozzle’s X-Y motion to form layers of porogen. The size of the strut is the most important factor to control the quality of

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<th>Table I</th>
<th>Factors for flow rate analysis design</th>
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<tr>
<td>Factor</td>
<td>Nominal values</td>
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<td>Pressure of the controller (psi)</td>
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<tr>
<td>Temperature (°C)</td>
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<td>Pressure of the reservoir (psi)</td>
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<th>Table II</th>
<th>List of three input parameters for eight-run factorial experiment</th>
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<td>Effect</td>
<td>Controller pressure (Pc, psi)</td>
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porogen, since strut size controls the pore size, and strut size variability leads to variation in the porosity of the scaffold. The size of the strut is a function of many process parameters such as the pressures of the valve controller and reservoir, temperature of the reservoir and the moving speed of the nozzle. Since limited time was available for full experimental characterization of all parameter interactions, DOEs was used to plan and analyze an eight-run fractional experiment for the strut size analysis (Table III). The nominal parameter settings are listed in Table IV. Four factors representing the most important settings for the size of the printed strut: the pressure of controller, temperature and pressure of the reservoir and the speed of the nozzle, were selected for further investigation.

2.5 Cell culture
The biocompatibility of the PCL scaffold was assessed by Osteoblast cell line (7F2) isolated from mouse bone marrow (ATCC, CRL-12557). 7F2 cells were routinely maintained in Alpha minimum essential medium without ribonucleosides and deoxyribonucleosides with 10 percent (v/v) FBS, 2 mM L-glutamine, and 0.5 percent (v/v) pen-strep (stock solution 10,000 units/ml penicillin and 10,000 µg/ml streptomycin) at 37°C in a 5 percent CO2 incubator (Li et al., 2005). Briefly, 7F2 cells were seeded on the scaffolds coated with 20 µg/ml collagen type I with a suspension of 1 million 7F2 cells/ml for 3 h on an orbital shaker (Belly Dancer, Stovall, Greensboro, NC, USA). Following seeding, the initial level of cell seeding was assessed by the Alamar BlueTM (BioSource, Camarillo, CA, USA) assay. In order to evaluate cell growth on the PCL scaffolds the Alamar BlueTM assay was performed again on the same samples at days 2, 4, and 6 post-seeding. Subsequently, the samples were fixed in 10 percent neutral buffered formalin (Fisher) for 1 h at room temperature and stored in phosphate buffered saline (PBS) at 4°C until cytological staining. For staining, the samples were washed once more with PBS and incubated with PBS containing 2 µg/ml nuclear stain-Hoechst 33258 (Bisbenzimide, Sigma, New York, NY, USA), and 1 µg/ml cytoskeleton stain-rhodamine phalloidin.

2.6 Scanning electron microscopy
The microstructures of cell cultured scaffolds were characterized by scanning electron microscopy (SEM) according to standard protocol (Li et al., 2005). Cell-seeded scaffolds were fixed in 2.5 percent aqueous gluteraldehyde for 1 h at room temperature then overnight at 4°C, dehydrated through graded alcohols, and dried with a critical point dryer (SPI Supplies, West Chester, PA, USA). All samples were then examined with a scanning electron microscope (XL-30 Environmental SEMFEG) using an acceleration voltage of 10.0 kV.

3. Results and discussion
3.1 Preliminary result of porogen fabrication system
Since SFF technology is able to form high precision real models combining with 3D reconstruction based on CT and MRI data, it has become a preferred way to generate defined porous structures. The most important task of this work was to develop a versatile SFF-based fabrication system. We started with the nozzle jet system selection and installation, which includes the nozzle, the reservoir and the heating element of the nozzle and reservoir. A SFF-based manufacturing system has been studied and developed to build a sucrose skeleton which serves as the bone scaffold porogen. Then PCL was extruded and cast into the sucrose porogen to form bone scaffolds with predefined structures and sufficient mechanical strength.

The design and integration of our porogen fabrication machine has been accomplished (Figure 2), and the porogen structure model has been designed. Figure 5(a) shows the printed sucrose porogen from the SFF porogen fabrication system. A CAD design with 500 µm struts and 500 µm pores was fabricated, using a motor speed of 0.1 m/s, controller pressure of 30 psi, reservoir pressure 90 psi and temperature 90°C, based on the DOEs results and the porogen design. The porogen pore diameter varies from 250 to 500 µm and the smallest strut size on the porogen is about 250 µm. Molten PCL was successfully injected into the porogens to create porous scaffolds with ovoid pores (Figure 5(b)). Scaffold fabrication was successful, producing a porous connected PCL scaffold with good structural integrity. Some parts of the injected scaffolds collapsed due to the softening of the porogen (about 30 percent), and some fissures are visible in the solidified PCL. So when injecting the scaffolds, the temperature of the scaffold material is critical and must be carefully monitored.

Room temperature solvent casting of scaffolds has also been explored, using poly(1actic-co-glycolic acid) in acetone and PCL in acetic acid. Porous structures were generated without any porogen collapse, but were too fragile to be used for bone scaffolding.

3.2 Selected porogen materials for designed fabrication system
The modified sucrose as a porogen material in terms of its biocompatibility, bioreabsorbability, mechanical strength, and manufacturability was evaluated in our preliminary study. For this, the porogen material was first melted until reached a semiliquid state and then extruded through a nozzle to form one layer of the part. In order to extrude the porogen material, a sucrose mixture was developed, which consists of sucrose,
honey, alum and butter in a weight ratio of 90:4:5:1. This mixture has met the requirements for our porogen material with desired melting temperature 80°C and injectable viscosity. Alum (aluminum sulfate, Al₂(SO₄)₃) can reduce the melting temperature of the mixture. Honey can also reduce the melting temperature and increase the hardness and the viscosity of the mixture. Butter will improve the lubrication of the mixture.

3.3 Viscosity measurement
The viscosity of sucrose mixture was measured at temperatures of 110, 120 and 135°C and shear rates ranging from 220 to 2200 s⁻¹. The viscosity of the melted sucrose solution decreases with increasing shear rate and increasing temperature (Figure 6). The decrease in viscosity with shear rate is referred as shear thinning and can be generally modeled with a power law. The viscosity is also highly dependent on the temperature. We used Matlab to determine the parameters to fit the viscosity data to a power law:

\[ \eta = H(T)k\gamma^a \]  

where \( \eta \) is the viscosity, \( H(T) \) the fluid material temperature, and \( \gamma \) the shear rate. Best fit constants for the measured data are \( a = 0.6402, b = 4361.2 \text{ cP}, T_0 = 56.67^\circ \text{C}, a = 634.9^\circ \text{C}, \) and \( T_a = 135^\circ \text{C} \) (Figure 6). The analyzed result will be used to model this nozzle system. These viscosity test results are important for controlling the parameter settings and to improve the quality of our fabricated porogen.

3.4 Result of flow rate measurement
To optimize the nozzle moving speed and estimate the manufactured part building volume, the determination of deposited materials flow rate is necessary. Experiments were conducted to measure the flow rate of the sucrose mixture at various parameters to understand the controllability of deposition of sucrose mixture and to improve the quality of the printed porogen. Mass of the deposited solution was measured on an electronic balance over a 20-s interval, and the average mass flow rate calculated. Volume flow rate was calculated by dividing the mass flow rate by the solution density 1,446 kg/m³, which was measured by weighing a filled 3-cc syringe. At the nominal parameter values, an average volumetric flow rate of \( 1.21 \times 10^{-6} \text{ ml/s} \) was calculated.

3.5 Results of variable analysis
Variable analysis results of flow rate
The flow rates from the eight-run experiments as well as that from the central run with nominal values are summarized in Table III. Flow rate was significantly different for each run (two-tailed t-test, \( p < 0.01 \)), except for runs 1 and 2, in which micronozzle controller pressure varied, and reservoir temperature and pressure were low. With the ten-step design for experiment analysis, factor and interaction effects were estimated in least square sense. The main factor effect of reservoir pressure \( (X_3) \) topped the rank list of factors. This means that the reservoir pressure was dominant to determine the flow rate. The second most important factor is the temperature of sucrose mixture \( (X_2) \).
The pressure of the micronozzle controller (X1) almost had no effect on the flow rate.

The ordered data plot for the eight experiment runs (Figure 7) demonstrates that the maximum flow rate is achieved at the higher temperature, higher reservoir pressure and controller pressure, and minimum flow rate is achieved at the lower temperature and pressures. Since the four largest flow rates (1.141, 1.245, 1.66, and 1.763) have factor X3 at +1, and the four smallest flow rates (0.795, 0.795, 0.93 and 1) have factor X3 at –1, X3 is the most important factor. The scatter plot (Figure 8), half normal probability plot, and rank list of main factors and interactions also demonstrate that factor X3 is the most important factor, X2 is the less important factor and the X1 is the least important factor. The interactive effects matrix suggests that the interaction between reservoir pressure and temperature appears to be more important than controller pressure and its interactions with the other factors. The contour plot for the flow rate as a function of the two dominant factors (Figure 9) shows the effects of temperature of the sucrose mixture and the pressure of reservoir are linearly related.

With a two-level three-factor fractional orthogonal DOEs, the uncertainty due to parameter setting in the flow rate analysis of a micronozzle system was evaluated. The sensitivity analysis using DATAPLOT made it possible to locate two dominant factors which had the greatest impact on the flow rate of the micronozzle. This gave us valuable information to improve the quality of our printed structures.

**Variable analysis results of strut size**
The variable setting of the four selected factors for strut sizes variable analysis are listed in Table V. The strut sizes measured by SEM from the eight-run experiments are summarized in Table VI. Following the same steps and procedures for flow rate analysis, factor and interaction effects were estimated.

The nozzle moving speed was the main factor to determine the strut size. The lesser important factors are the pressures of controller and reservoir. One goal of this study was to identify process parameter settings to minimize strut size in order to create higher-quality porogens and smaller scaffold features. It has been found from Table IV that the minimum strut diameter in this study was 200 μm. The DOE identified that higher nozzle moving speed, lower temperature of the sucrose and smaller reservoir and controller pressures can minimize strut size (Lu, 2008). However, nozzle moving speed is limited considering machine vibration at high speed, which will reduce the quality of the printed porogen (Tables V-VI).

### 3.6 Biocompatibility tests

The mechanical properties and degradation rates of PCL and PCL/CaP composite biomaterial scaffolds have been performed in previous studies and their potential application in hard tissue engineering was demonstrated (Mondrinos et al., 2006). Since new porogen building materials were introduced in this work, the cell-biomaterial interactions of resultant scaffolds were assessed. Osteoblasts, the cells that synthesize the mineralized extracellular matrix tissue that gives bone its strength, were cultured on sucrose-molded PCL scaffolds. A mouse osteoblast cell line (7F2, ATCC) was used to model osteoblast-scaffold interactions. The results show that 7F2 cells were able to attach to the PCL scaffolds (Figure 10(a) and (c)), and that osteoblasts grew well and they were confluent after four days of post-seeding on PCL scaffolds (Figure 10(b) and (d)). The morphology of 7F2 cells growing on PCL scaffolds was examined by SEM (Figure 11). The cells flatten on the surface, and SEM images confirm that the cells completely cover the biomaterial surface after four days post-seeding. The cell metabolic activity/cell...
The normalized cell proliferation data indicated that the cell growth at days 4 and 6 were significantly enhanced compared to day 2 ($p < 0.05$). The AB data were corroborated qualitatively by the observed increase in the density of Hoechst 33258-stained nuclei following four days of post-seeding culture in vitro on the scaffolds (Figure 10). We note that at this time point, cells were visibly growing both on the struts and the interior pore structures of the scaffolds investigated (Figure 10(b)).

The results compared quite favorably to metabolic activity of cells cultured on PCL scaffolds cast in plaster porogens produced by another method (Lu, 2008), and similar to PCL/CaP composite scaffolds which have been shown in our previous studies to be more biocompatible than pure PCL scaffolds (Mondrinos et al., 2006). These results confirm the potential advantage of using more biocompatible porogen materials in the designed SFF machine.

### 4. Conclusion

A new SFF-based manufacturing system for building sucrose bone scaffold porogens has been developed. This research integrates SFF, systems and control, and tissue engineering in one intelligent system for structured, highly porous biomaterials bone scaffolds. The implementation of the EFD pressure-based extrusion will allow for the use of sucrose solutions with viscosity high enough to achieve solidification in an acceptable time frame for layer by layer fabrication. The fabrication process for PCL scaffolds has been developed and tested using the newly developed porogen system. The resultant scaffolds demonstrate the defined porous structure designed into the sucrose porogens. The sucrose mixture composition was optimized for short solidification time and low viscosity. Viscosity measurements of the sucrose mixture demonstrated shear-thinning behavior and strong temperature dependence, and viscosity data were fit to a power law with Arrhenius temperature dependence. The effects of process parameters on the nozzle flow rate and porogen strut size were studied and analyzed using the DOEs method to identify key dominant parameters and parameter interactions. It has been found out that the reservoir pressure was the most dominant factor to determine the flow rate, followed by the sucrose temperature; and the build...
Figure 10 Overlay of bisbenzimide and rhodamine phalloidin staining of 7F2 cells cultured on PCL scaffolds, 5× or 10× objective magnification

Notes: Cell nuclei stain light blue, and cytoskeletal protein stains pink. (a, b) day 2 after seeding shows initial attachment, with some areas of bare scaffold material (arrows) and (c, d) at day 4, the cells were confluent

Figure 11 SEM images of 7F2 growing onto the PCL scaffolds

Notes: (a) Flattened 7F2 cells on PCL scaffold at day 1, 1,000×; (b) day 1, 200×; (c) day 4, 200×
Figure 12 Normalized increase in Alamar Blue™ reading over the six-day in vitro culture period following the initial 24 h seeding period for PCL scaffolds. Metabolic activities as measured by Alamar Blue™ taken immediately following the 24 h seeding period. y Error bars represent the standard deviation from the mean for each sample (n = 5). *Statistically significant differences (p < 0.05) compared to each two days by one-way ANOVA with Tukey-Cramer post-tests for multiple comparisons.

nozzle moving speed was the dominant factor affecting strut size. These analyses guided us in improving the quality of porogen fabrication.

The biocompatibility of fabricated PCL scaffolds using the newly developed sucrose-based porogen system was assessed in vitro. The sucrose molded PCL scaffolds showed cell attachment, cell proliferation to confluence, and increasing metabolic activity over four to six days. Comparing with scaffolds fabricated with a plaster-based porogen material, the sucrose-porogen made scaffolds showed better increases in metabolic activity and cell proliferation. The results suggested that the sucrose porogen-based fabrication method has advantages in bone tissue engineering.

References


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