Rapid freeze prototyping technique 
in bio-plotters for tissue scaffold fabrication

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Abstract
Purpose – The purpose of this paper is to develop a new bio-platter using a rapid freeze prototyping (RFP) technique and to investigate its potential applications in fabricating tissue scaffolds.

Design/methodology/approach – The development of cryogenic bio-plotters including design steps of hardware as well as software is addressed. Effects of structural parameters and process parameters on the properties of tissue scaffolds are demonstrated through simulation and experimental results.

Findings – The paper finds that the RFP method is suitable to fabricate macro- and micro-porous scaffolds, especially for temperature-sensitive polymers. In addition, through simulation and experiment results, it also shows that macro- and micro-porous properties could be manipulated by structural parameters and process parameters, respectively.

Research limitations/implications – This paper shows that the chamber temperature is an important process parameter that can provide the means to control the micro-porous structure of the scaffold. However, if the temperature is set too high, the fiber is frozen so rapidly that it cannot be fused with other fibers of the previous layer. On the other hand, if the temperature is too low, the fiber is not solidified fast enough. So, the chamber temperature, together with extruding pressure and nozzle velocity, must be optimized, which will be further investigated in future work.

Originality/value – The RFP technique is successfully proposed to construct 3D tissue scaffolds. In addition, a new cryogenic bio-plitter is designed and developed, in which general algorithms of rapid prototyping method are presented and implemented, facilitating the fabrication of tissue scaffolds with various cross-hatching patterns in a RFP process.

Keywords Rapid prototypes, Cryogenic equipment, Heat engineering, Programming and algorithm theory, Cytology

Paper type Research paper

1. Introduction

Tissue engineering (TE) merges the fields of cell biology, engineering, material science and surgery to generate new functional tissue using living cells and a matrix or scaffolding method (Langer and Vacanti, 1993). It involves the development of methods to build biological substitutes as supplement to whole organ or tissue transplantation. Matrices playing a central role in TE are utilized to deliver cells to desired sites in the body. Therefore, the fabrication of these specifically designed porous structures, i.e. scaffolds, for application in TE is one of the major foci in current biomedical research.

There have been several efforts to produce scaffolds with 3D pore inter-connectivity using different processing techniques, such as fibre bonding, solvent casting, particulate leaching, melt moulding, and membrane lamination (Yang et al., 2001). For most of these processes, resultant scaffolds have unpredictable pore sizes and arrangements. With the introduction of rapid prototyping (RP) technology, scaffolds suitable for TE can be produced with 3D structures containing consistent pore sizes and arrangements, which leads to significant amounts of work fabricating TE scaffolds using RP methods (Hollister, 2005; Leong et al., 2003; Yang et al., 2002). Existing RP systems that can be used for TE scaffolds are categorized by processing techniques including: laser-based processing systems which photopolymerize a liquid in stereolithography (STL) systems (Chu et al., 2001), or sinter powdered material in selective laser sintering systems (Tan et al., 2005); printing-based systems, e.g. 3D printing systems and wax-based systems, which print a chemical binder onto a powder bed and print two types of wax material in sequence (Yeong et al., 2006), respectively; and nozzle-based systems, e.g. fused deposition modelers and bio-plotters, which deposit material that is processed either thermally or chemically through a nozzle (Ang et al., 2006).

Although most commercial RP systems can build complex external shapes to match human anatomy with complex porous micro structures from bio-compatible materials, the ultimate fabrication technology must be able to fabricate hybrid biomaterial and cell/gene/protein structures over a...
range of size scales. Among the above systems, the bio-plotting technology emerges as a potential method to fabricate a wide range of materials including various biomaterial systems and even living cells (Landers et al., 2002; Xiong et al., 2002; Yan et al., 2003; Woodfield et al., 2004; Liu et al., 2007). Generally, the bio-plotter technology is potential to build complex, porous structures matching human anatomy from multiple bio-compatible materials with high resolutions. One of the most advanced bio-plotters has been developed at the Freiburg Materials Research Centre (Landers et al., 2002). A unique feature of this bio-plotter is that materials can be printed into a reactive support medium, which serves to support overhanging structures through buoyancy forces and to contain chemicals that will react with the printed material. Currently, this type of bio-plotters is often employed to fabricate tissue scaffolds.

The objective of this paper is to describe the development of a novel TE fabrication method using a cryogenic prototyping (CP) technique. The CP method is similar to the rapid freeze prototyping (RFP) that has been introduced to build ice patterns with good accuracy and surface finish for investment casting (Zhang et al., 1998, 2000; Sui et al., 2000; Peng et al., 2007). The key innovation in the CP method is its ability to control the macro- and micro-architecture of the TE scaffold via manipulating the design and low-temperature processing parameters of the plotter and the chamber. When this method is employed to fabricate TE scaffolds, preliminary results have shown that it is able to produce pre-determined scaffold with open pore networks, which facilitates nutrients and cells to pass into the scaffold while ensuring waste products can be transported away.

2. Cryogenic prototyping

In the CP method, which is different from conventional RP methods, the deposited material is solidified in a cryogenic chamber to avoid collapsing of scaffold structures. In addition, the CP method is able to produce pre-determined scaffolds with controlled macro- and micro-porous structures. This is because the material solutions are dispensed in controlled paths and rapidly frozen to retain the deposited structures, hence forming the macro-structure of the scaffold. Subsequent removal of ice crystals formed during the CP process with a controlled freeze drying process will result in controlled micro-porosity of the scaffold. Furthermore, this method allows the fabrication of temperature-sensitive polymeric scaffolds, which often cannot be easily processed using conventional RP methods because of the involvement of elevated processing temperatures. Generally, two significant advantages of CP are as follows:

1. the unique and novel method to control the formation of crystalline structures by rapidly frozen solvents to manipulate the formation of micro- and nano-sized pores; and

2. the avoidance of porogens in processing.

The schematic diagram of a prototype cryogenic bio-plotter that is based on the CP method and its process flow is shown in Figure 1. The nozzle has a translational motion in vertical direction. In combination with a 2-degree of freedom moving platform on a horizontal plane, 3D scaffolds can be built up layer by layer. The flow of materials is controlled by a pneumatic-based controller. In addition, the material is dispensed in a cryogenic chamber, in which the temperature is critically controlled and maintained by a refrigerated circulator.

3. Development of CP

The development of CP includes the design of both hardware as well as software. In order to shorten the development time, a 3D dispensing system of Shotmaster 300-3 A with a resolution of 0.01 mm is employed to realize 3D motions and to extrude materials at preset pressures. The maximum swept volume is 300 × 300 mm on the horizontal plane and 80 mm in the vertical direction. It is also equipped with a customised refrigerator of the PolyScience family that is able to maintain a temperature range from –45 to 200°C.

3.1 Cryogenic chamber

A cryogenic chamber is designed to maintain a steady set point of temperature within the swept volume of the CP plotter. Basically, it has two components: a refrigerated circulator and a well-insulated chamber. The liquid in the circulator bath is cooled and circulated through external coil loops of the chamber to control and maintain the temperature of the insulated chamber. The coils are made of stainless steel to eliminate corrosive effects of the fluid.

3.2 RP technique

Once the hardware is fully built, assembled and set up, the usual RP techniques are incorporated in the process to fabricate the TE scaffolds. The RP process to build tissue scaffolds is previously shown in Figure 1. There are three major components: the Computer-Aided Design (CAD) components, the Computer-Aided Manufacturing (CAM) components and the dispensing systems. In the CAD system, 3D objects are designed or modeled from data generated with magnetic resonance imaging, Computer Tomography or X-ray. The data files are then sent to the CAM systems to be converted into 2D data via slicing and hatching algorithms. Subsequently, these slice data are serially transformed into control signals to a controller of dispensing system for the building of the scaffolds.

The typical exchange data between the CAD and CAM systems are often in a STL format, which is a list of triangular facets representing external surfaces of the model (Jacobs, 1996). This format has become the de facto industry standard and is available for most commercial CAD systems, e.g. SolidWorks. With the hardware as selected and designed, there is a need to transfer the CAD data to the dispensing system. As such, a CAM program that is suitable for building TE scaffolds needs to be developed. Basically, it must possess the basic functions as shown in Figure 2, i.e. accept the data in the STL format, appropriately slicing the 3D model into suitably selected layers, creating the 2D slices for the dispensing system and determine the porosity of the resulting scaffold.

3.3 RP algorithms

3.3.1 Decoding STL files

The STL file is often specified as a binary format. Binary STL files consist of a 80-b header line that can be interpreted as a comment string. The following 4b interpreted as unsigned long integer give the total number of facets. What follow are a normal and three vertices for each facet. Each coordinate is
represented as a 4-b floating-point number (IEEE Standard 754). There is a 2-b spacer between each facet. The result is that each facet is represented by 50 b including 12 b for the normal, 36 b for the three vertices, and 2 b for the spacer. If \( n \) is the number of facets, the file size is calculated as:

\[
\text{STL file size} = 84 + 50n \text{ (bytes)}
\]

For IEEE floating-point numbers, they have three basic components: sign, exponent, and mantissa. The mantissa is composed of the fraction and an implicit leading digit. Table I shows the layout for single (32-bit) precision floating-point values. The number of bits for each field is shown (bit ranges are in square brackets).

\[
\begin{array}{c|c|c|c|c}
\text{Sign} & \text{Exponent} & \text{Fraction} & \text{Bias} \\
1 [31] & 8 [30-23] & 23 [22-00] & 127 \\
\end{array}
\]

With these components, the value of an IEEE-754 number is computed as:

\[
\text{Decimal value} = \text{sign} \times 2^{\text{exponent}} \times \text{mantissa}
\]

By repeatedly calculating all bytes in STL files, three vertices and the normal vector of every facet can be presented in a decimal system.

### 3.3.2 Slicing 3D objects
Once the data of solid objects are regenerated, which is a set of triangles \( m \), slice planes and slice thickness need to be defined. Intersection between slice planes and facets results in a set of segments constituting contours. In bio-plotters, the slice thickness, \( h \), depends on the size of the nozzle, and therefore is constant. Because the object can be freely rotated, without losing generality, the slice axis that is perpendicular to the slice plane is assumed to be the \( z \)-axis.

The flowchart to determine the intersecting segments between the facets and the slice planes is shown in Figure 3. The intersection is mainly based on algorithms of determining the signed distance from a point to a plane, which side of the plane that the point is on, and of finding the intersection between a segment and a plane.

### 3.3.3 Hatching 2D slices
The slice, which is derived from the slicing algorithm, composes of discrete segments. Owing to the fact that there
In order to identify projections and holes, i.e. areas that need to be filled and areas that should not be, the rule based on the hierarchy of the closed loops (contours), which is computationally expensive, is often employed. That is if a contour is surrounded by \( n \) contours and \( n \) is an even number, the contour represents a protrusion and should be filled. On the other hand, if it is odd, it is a hole and thus should not be filled (When \( n \) is zero, it is a protrusion) (Tata et al., 1998). In this paper, a more efficient approach to hatch the slice without determining the hierarchy of the contours is presented.

The method is based on the following observation: a straight line can interact with the contour in two ways, the line either misses the contours completely or meets the contours in an even number of points which, in order, are I, O, I, O, ..., I, O as shown in Figure 4. Thus, a set of segments that join the first point to the second one, the third to the fourth, the fifth to the sixth and so on, i.e. from I to O, define the hatch pattern of the slice. With this observation, a hatching algorithm has been implemented as shown in Figure 5, in

Figure 3 Procedure of creating slices

Input: \( m \) facets, thickness \( h \)

Scan ranges: \( z_{\text{min}}, z_{\text{max}} \)

\( z = z_{\text{min}} \)

Define a plane passing \( z \)

\( z = z + h \)

Create a new slice; \( i = 1 \)

Read facet \( i \)

Determine: plane \( \cap \) facet

No

Is it a segment?

Yes

Add it to the current slice

If \( (i = m) \)?

No

Yes

If \( (z \geq z_{\text{max}}) \)?

Yes

Return slices

No

Figure 4 Hatching a polygon with lines

cannot be a solid without thickness, the segments invariably form closed and non-intersecting loops identifying holes and projections. Projections should be hatched with a pattern of equally spaced straight lines that are parameterized by an appropriate raster angle and a raster gap and holes are not hatched, as shown in Figure 4.

Figure 5 Procedure of creating hatch patterns

Input:
- hatch parameters: \( \alpha, g \)
- current slice: \( S \)

Define a set of lines, \( L \supset S \)

Read \( l_i \in L, s_j \in S \ (i = j = 1) \)

Determine: \( l_i \cap s_j \)

No

Are they intersected?

Yes

Append it to an array, \( P \)

\( j = j + 1 \)

No

Is \( s_j \) the last?

Yes

\( i = i + 1 \)

Reset: \( j = 1 \)

No

Is \( l_i \) the last?

Yes

Sort the point array, \( P \)

Return hatch lines
which the main step is to determine the intersection of predefined lines and the segments of the slice.

### 3.3.4 Computing porosity
These algorithms assure that the architecture of the scaffold defines the ultimate shape of the target tissue. Apart from material issues, a good tissue scaffold must comprise an interconnected, open pore network allowing nutrients and cells to pass into the scaffold while ensuring waste products can be transported out. In this section, mathematical models are formulated to predict the porosity of the scaffolds under the influence of raster fill patterns, i.e. raster width and raster gap.

The porosity of a porous medium describes how densely the material is packed. It is the proportion of the non-solid volume to the total volume of material, and is defined by the ratio:

\[
P_{\text{calc}} = 1 - \frac{V_t}{V_s} \tag{3}
\]

where \( V_t \) = scaffold fiber volume; \( V_s \) = total scaffold volume.

The slice area can be computed from slice data, e.g. polygon, resulted by the slicing algorithm; and the fiber volume is determined by segments resulted from the hatching algorithm. Approximately, the volumes of the scaffold fiber and the total scaffold can be computed as follows:

\[
V_t = \sum_{i=1}^{n_t} \pi r_i^2 l_{i,i} \tag{4}
\]

\[
V_s = \sum_{i=1}^{n_t} V_{s,i} = h_s \sum_{i=1}^{n_t} A_{h,i} \tag{5}
\]

where \( n_t \) = the number of layers; \( r_i \) = the radius of fibers; \( l_{i,i} \) = the total length of fibers in the \( i \)th layer; \( h_s \) = the slice thickness; \( A_{h,i} \) = the area of the \( i \)th layer.

In studying the deposition of a water drop on a cold plate, the authors have shown that the smaller the droplet radius, the rounder its cross-sectional shape. If the radius is less than 1 mm, the cross-sectional profile is almost circular (Sui et al., 2000). In experimental conditions of the bio-plotters, the radius of the nozzle is often much less than 1 mm. Thus, the radius of fibers could be approximately a half of the slice thickness. As a result, substituting equations (4) and (5) into equation (3) results in:

\[
P_{\text{calc}} = 1 - \frac{n_t h_s \sum_{i=1}^{n_t} l_{i,i}}{4 \sum_{i=1}^{n_t} A_{h,i}} \tag{6}
\]

With equation (6), the porosity of any scaffold structures designed in this system can then be computationally predicted.

### 3.3.5 Converting hatching data to machine commands
Once the scaffold structure and its porosity are satisfied, the path data are converted and stored in a file which contains the program commands. These commands are positional codes and auxiliary codes interpretable by the MuCAD, which is the basic control for the fluid dispensing system Shotmaster 300-3A. The path data can be partly or fully exported for consecutive layers.

### 4. Simulation and preliminary results

#### 4.1 Simulation results
A version of CAM Software, so named Computer-Aided Tissue Scaffolds (CATS), has been developed. For demonstration purposes, a cylindrical object with the outer diameter of 10 mm, the inner diameter of 4 mm, and the height of 3 mm is designed in CAD Software, SolidWorks, and then saved in binary STL format. The solid object is successfully regenerated in CATS as shown in Figure 6.

With this software, the pattern for the dispensing path in slices can be selected and the dispensing process can be checked via simulation. Figure 7 shows a simulated scaffold in a 0/60/120° pattern, in which the fibers are oriented at 0, 60, and 120° with respect to the horizontal in the first slice, the second slice, and the third slice, respectively.

The porosity of the cylindrical object can be changed by varying the raster gap settings. Simulations with raster gaps of 0.06, 0.12, 0.18, and 0.24 mm with the same lay-down pattern (0/60/120°) and raster width (0.2 mm) show that a higher raster gap results in a better porosity. Similar trend is also observed as the scaffolds are formed with other raster widths.

Using equation (6), the predicted values of porosity with respect to the raster gap and the slice thickness are calculated and shown in Figure 8. These simulation shows that:

- the porosity is more sensitive to the raster gap in lower ranges; and
- with the same raster gap, a smaller slice thickness, i.e. thinner fibers, leads to a higher porosity.

#### 4.2 Preliminary experimental results
Experiments were carried out using 4 percent wt/vol chitosan solution (in 2 percent acetic acid). The temperature within the chamber was maintained at -16°C. The fibers were extruded through a nozzle of 0.5 mm diameter under an applied pressure of 8.0 kPa. The nozzle was moved at the speed of 5 mm/s. Figure 9 shows a scaffold sample of the cylindrical object modelled in Figure 6. Visually, its overall shape matches the desired solid object.

The 3D macro-pore connectivity of the sample is shown in Figure 10. The result was obtained using a scanning electron microscopy JSM-5600LV. The figure shows that the pores are quite well interconnected. The macro porosity is further shown in Figure 10 where fibers of 0.5 mm diameter maintain a relatively uniform gap of 0.3 mm.

In order to verify the cross-sectional profile of the fibers the scaffold was sliced. Figure 11 shows the cross section of two fibers and that the cross-sectional profile is almost circular as assumed in Section 3. Moreover, the micro-pore structures of the fibers are also shown in Figure 12. This was formed by the phase separation and the sublimation of solvent during the freeze-drying process. The micrographs show that there are porous channels within the fibers and they are locally aligned. These porous channels are found sensitive to changes of the chamber temperature. At a higher temperature, the rate of freezing is slower, resulting in formation of larger ice crystals that relates to larger pore sizes after freeze drying. Therefore, chamber temperature is an important process parameter that can provide the means to control the micro-porous structure of the scaffold. However, it was noted that if the temperature is set too high, the fiber is frozen so rapidly that it cannot be fused with other fibers of the previous layer. This will result
in a fragile structure. On the other hand, if the temperature is too low, the fiber does not solidify fast enough. As a result, the fiber could collapse. As such, the chamber temperature must be optimized so that it is able to achieve not only desired micro-pore sizes but also highly interconnected macro-pore structures.
Figure 10 Macro porosity

Figure 11 Circular profile of fibers

Figure 12 Section views of micro porosity

(a) Cross section

(b) Longitudinal section
5. Conclusion

In this paper, general algorithms of RP method have been presented and implemented. These algorithms are to facilitate the fabrication of tissue scaffolds with various cross-hatching patterns in a RFP process. In addition, the formula to predict the macro porosity of the structure has been derived, which helps to optimize structural parameters in order to obtain desired macro-pore inter-connectivity. Experimental results have shown that the RFP method is suitable to fabricate macro- and micro-porous scaffolds, especially for temperature-sensitive polymers. Given dispensed materials, the micro-porous property of the scaffold are believed to be controllable by optimizing process parameters including chamber temperature, extruding pressure and nozzle velocity, which will be further investigated in our future work.

References


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