Rapid Prototyping of 3D Scaffolds for Tissue Engineering Using a Four-Axis Multiple-Dispenser Robotic System

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Abstract

A desktop rapid prototyping (RP) system has been developed to fabricate scaffolds for tissue engineering (TE) applications. The system is a computer-controlled four-axis machine with a multiple-dispenser head. This paper presents the scaffold fabrication process to build free-form scaffolds from relevant features extracted from given CT-scan images for TE applications. This involves obtaining the required geometric data for the scaffold in the form of a solid model from CT-scan images. The extracted scaffold model is then sliced into consecutive two-dimensional (2D) layers to generate appropriately formatted data for the desktop RP system to fabricate the scaffolds. The basic material processing involves the sequential dispensing of two or more materials to form a strand. The four-axis system enables strands to be laid in a different direction at each layer to form suitable interlacing 3D free-form scaffold structures. The multiple-dispenser head also allows the introduction of living cells and additional materials during the scaffold building. The building of the scaffolds with the desktop RP system is described based on the sequential dispensing of chitosan dissolved in acetic acid and sodium hydroxide solution. Neutralization of the acetic acid by the sodium hydroxide results in a precipitate to form a gel-like chitosan strand.

Keywords: Scaffold; Rapid prototyping; Tissue engineering

1. Introduction

In tissue engineering (TE), scaffolds built from synthetic or natural materials serve as temporary surrogates for the native cellular matrix. Rapid prototyping (RP) is suitable for tailoring individual patient-specific scaffold parts because of its flexibility to build complex structures. At present, several RP techniques have been exploited and adapted for generating individual TE scaffolds, such as fused deposition modeling (FDM) [1,2], laminated object manufacturing (LOM) [3], three-dimensional printing (3DP) [4], multiphase jet solidification (MJS) [5] and 3D plotting [6].

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This paper presents the development of a four-axis multiple-dispenser robotic system to fabricate scaffolds. The process involves the sequential dispensing of materials that coagulate to form inter-lacing strands for the building of the scaffold. An additional property is that the basic structures can be achieved without high temperature, unlike FDM. This enables fabrication with materials or material additives that will otherwise decompose under the high-temperature fabrication condition. It also facilitates the incorporation of proteins and living cells into the scaffold via additional dispensers. This feature makes the process more suitable for tissue engineering applications.

The focus is on the scaffold fabrication technology for TE. The mechanical and structural requirements of TE scaffolds and the pre-requisites for scaffold fabrication techniques are described. Emphasis is on the fabrication process using the robotic dispensing system to build scaffolds. This includes obtaining individual geometrical data to form 3D CAD model and segmenting the model to two-dimensional (2D) layers to generate data for the four-axis multiple-dispensing RP system to fabricate scaffolds automatically.

2. Materials and requirement for fabricating scaffold

2.1 Materials

As the scaffolds for tissue engineering will be implanted in the human body, the scaffold materials should be non-antigenic, non-carcinogenic, non-toxic, non-teratogenic and possess high cell/tissue biocompatibility, so that they will not trigger any adverse cellular reactions after implantation.

In this research, chitosan was used as the scaffold material. Chitosan, which is a naturally occurring amino-polysaccharide, is biodegradable, biocompatible and nontoxic [7]. A high-purity chitosan powder ($C_{12}H_{24}N_2O_9$) is used. The material was prepared by dissolving chitosan in acetic acid to form a hydrogel. The gel was contained in the plastic syringe barrel and dispensed by pressurized air. NaOH solution was used as coagulation and dispensed via another syringe using a motorized plunger.

2.2 Requirement

Besides material issues, the macro- and micro-structural properties of the scaffold are also very important [8, 9]. In general, the scaffolds require individual external shape and well defined internal structure with interconnected porosity.

Ideally, a scaffold should have the following characteristics:

(a). be highly porous with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste; (b) have suitable surface chemistry for cell attachment, proliferation, and differentiation; (c) possess mechanical properties to match those of the tissues at the site of implantation; (d) be easily fabricated into a variety of shapes and sizes and (e) possess interconnecting porosity so as to favor tissue integration and vascularity. [10, 11]

3. The process

RP is suitable for tailoring individual parts for specific applications and this has a great impact for the biomedical industry. RP already has areas of applications in building prosthetics and mechanical implant structures [12]. These computer models were produced by computer-aided design (CAD) software from computer tomography (CT) or magnetic resonance imaging (MRI) data. Considering the time, flexibility and accuracy requirements, RP technologies are very suitable for application in tissue engineering to fabricate scaffolds.

A general framework for the application of rapid prototyping in the area of tissue engineering is shown in Fig. 1 [13]. A specific area of the patient is scanned by computer tomography or magnetic resonance and the data are imported into a CAD software. The scaffold is designed according to the individual requirements using the CAD software and postprocessed data for the fabrication of the scaffold is then transferred to a RP system to produce the scaffold with a biocompatible and biodegradable material. Living cells are seeded onto the surface of the scaffold after or during the RP process. When the cell number increases following cell culture treatment, the scaffold is implanted into the human body and eventually replaced by natural tissue.

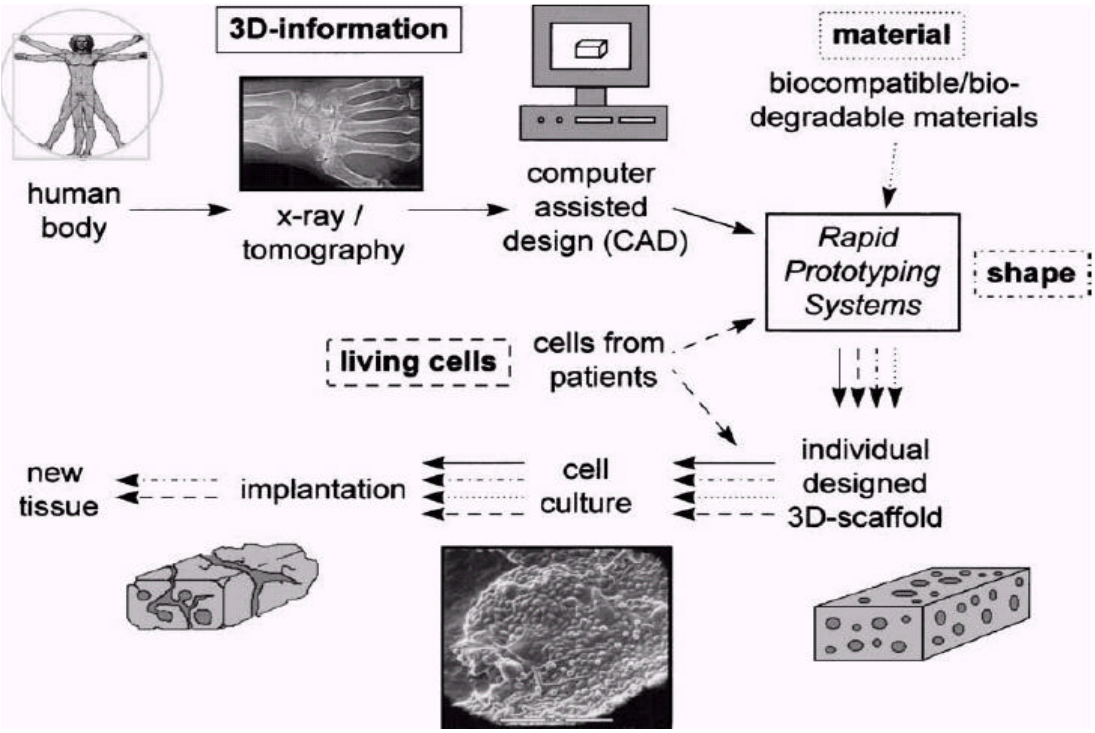


Figure 1. A framework of biomedical RP [13]

3.1 Input data

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) systems are the two most commonly used medical scanning systems. Through both the CT and MRI scan, a series of digitized gray-scale slice images of the scanned body is obtained. A suitable three-dimensional (3D) computer model is derived from the scanned images using the Materialise's

Interactive Medical Image Control System (Mimics) software [14]. The Mimics software is an interactive tool for the visualization and segmentation of CT / MRI images and 3D rendering of objects. The purpose of the data processing is to produce 3D reconstructions of objects directly from the digitized gray-scale image data and to convert the medical data to the data that can be processed by rapid prototyping systems. This involves separating the data of the tissue of interest from the scan data sets, or generating a certain part of the tissue from the available data. In some cases, the missing part of the tissue is extracted to create the implant for the scaffold building. Once the certain tissue part is separated or created, it can be converted into data formats that are compatible with RP systems, including Standard Triangulation Language (STL), Initial Graphic Exchange Specification (IGES), Standard for the Exchange of product Model Data (STEP), Common Layer Interface (CLI) and Virtual Reality Modeling Language (VRML), etc.

3.2 Robotic dispensing system

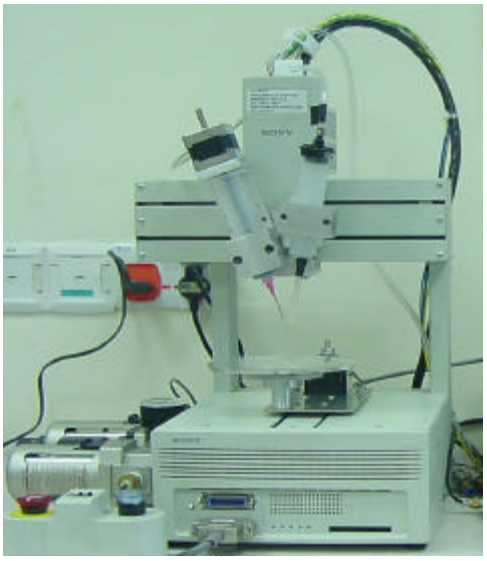


Figure 2. RPBOD system

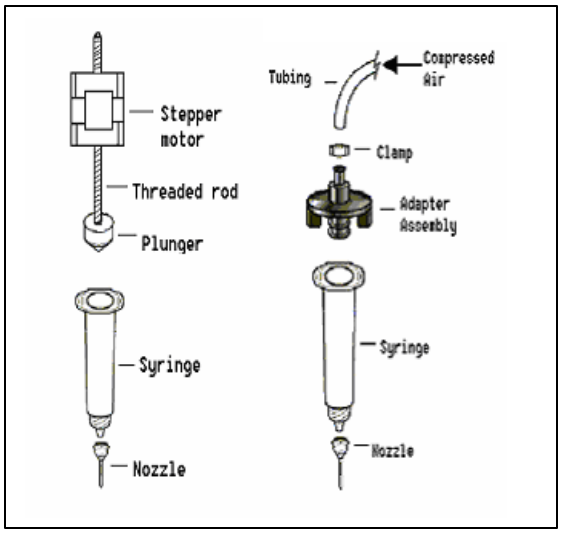


Figure 3. Mechanical & pneumatic dispenser

The rapid prototyping system shown in Figure 2 for the fabrication of scaffolds is a four-axis multiple-dispenser robotic system (RPBOD) based on the Sony Robokits. It is capable of three simultaneous translational movements along the X-, Y- and Z-axes with an added rotary motion about the Z-axis. The three translational movements have positioning accuracy of up to 0.05mm and a minimum step resolution of 0.014mm.

There are two kinds of dispensing mechanisms, pneumatic and mechanical (Figure 3). The pneumatically driven syringe dispenser is controlled by a solenoid-operated pneumatic valve. The mechanical dispenser is controlled by a plunger driven by a stepper motor. By controlling the displacement of the plunger, the dispensing rate can be precisely regulated, particularly at very low flow rate (such as 0.5 μl/sec).

The control software integrates the processes of slicing, which generates sliced layers in the +Z-direction, and dispensing, based on the slicing, to build suitable scaffold layer-by-layer.

3.3 Scaffold building

To generate a scaffold, the chitosan and NaOH are sequentially dispensed in each scan pass. The chitosan fluid was extruded and allowed to contact the base. The coagulation medium (NaOH) follows closely before the chitosan spreads out. The two materials react to precipitate into a strand. As shown in Figure 4, the tip on the right dispenses the chitosan and that on the left dispenses the NaOH solution, positioned approximately 5 mm apart. The dispense sequence is from left to right, and the nozzles are individually timed to dispense only when over the same section.

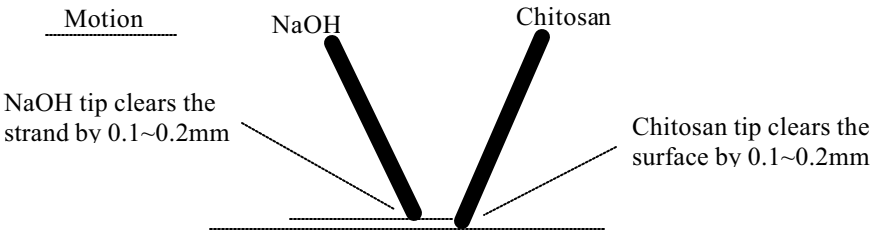
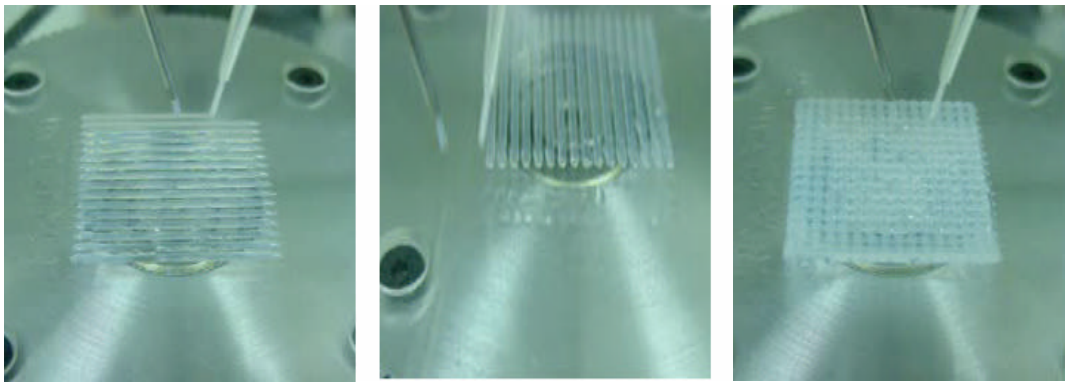


Figure 4. Nozzles position of twin dispensing

Figure 5 shows the process of scaffold fabrication by the dual dispensing method. During the dispensing process, the chitosan gel is dispensed as the dispenser moves (from left to right), leaving the chitosan gel on the base. Immediately following, the the mechanical dispenser drops the NaOH solution to precipitate the chitosan gel.



(a) **(b)** **(c)**

Figure 5. Scaffold fabrication process by dual dispensing

(a) Fabrication of first layer; (b) Start of dispensing for second layer;

(c) Scaffold building layer by layer.

After the first layer, the base is rotated by 90 degrees and the dispensers are lifted to a higher level that allows for the chitosan gel for the next layer to lay on the previous layer. The robot then generates the second layer similarly (Figure 5b) and the scaffold is progressively built as layers are sequentially generated in this manner (Figure 5c).

The chitosan scaffold built by this dual dispensing method exhibits excellent uniformity and strength. This has practically eliminated the occurrence of edge curling, the primary cause of strand dragging in chitosan scaffolding fabrication process. Edges of scaffolds are also better defined and good surface uniformity of the top layer is maintained (Figure 6). The process has good reproducibility, once properly calibrated [15].

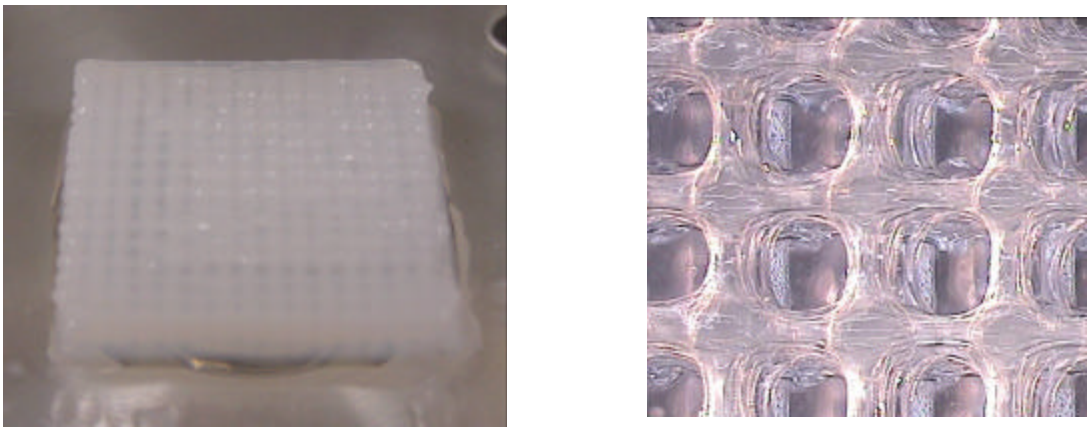


Figure 6. Freshly built chitosan scaffold and the air-dried scaffold under optical microscope (15X)

3.4 Generation of irregular shape scaffold

The advantage of RP technologies is their ability to produce complex 3D shape from a given computer model. As described earlier, the Mimics software enables scan data to be imported and the model of certain tissue part can be appropriately separated or generated, and subsequently converted into a data format that is compatible with the RP system. Figure 7 shows the model of a skull generated from its CT scan images. The bone has been separated from other soft tissues by setting a suitable threshold value. A 3D computer model of a patch has also been interactively created that can fill the hole by using editing and segmentation tools provided in Mimics. The model is then transferred in STL format to the RPBOD. Figure 8 shows the model displayed on the monitor of the RPBOD.

The model can be appropriately rotated before slicing in the Z-direction. The information of these layers is saved as CLI file, which is a simple, efficient and unambiguous format for data input to fabricate the model layer-by-layer. Figure 9 (a) and (b) show four consecutive scanned layers. The direction of the scan lines is set to intersect that of the preceding layer at 90 degrees. Hence, the built strands crossed at each layer to form the scaffold. The quality of built scaffold depends on the characteristics of the materials and experimental conditions, including the concentration, dispenser speed, and dispensing rate.

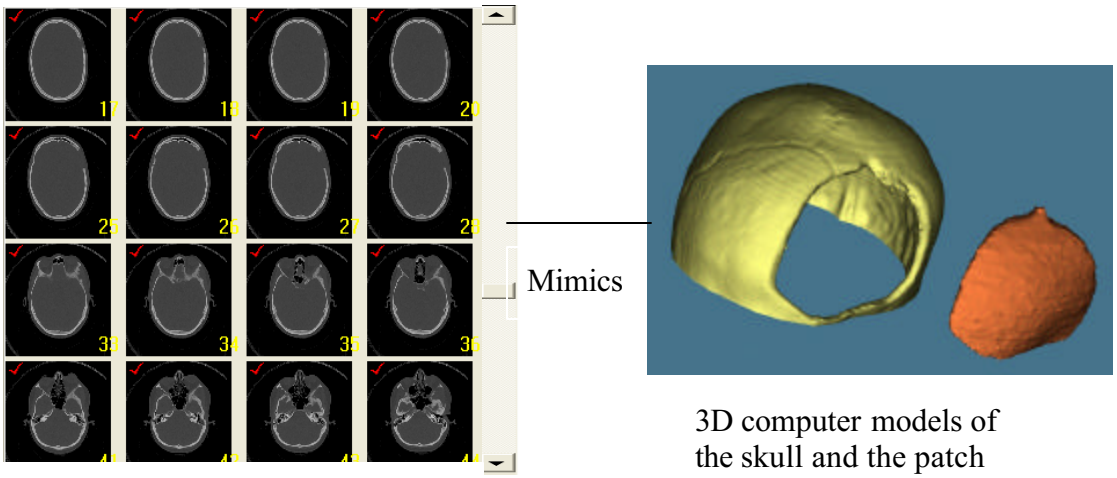


Figure 7. The conversion of CT images to 3D computer mode by Mimics.

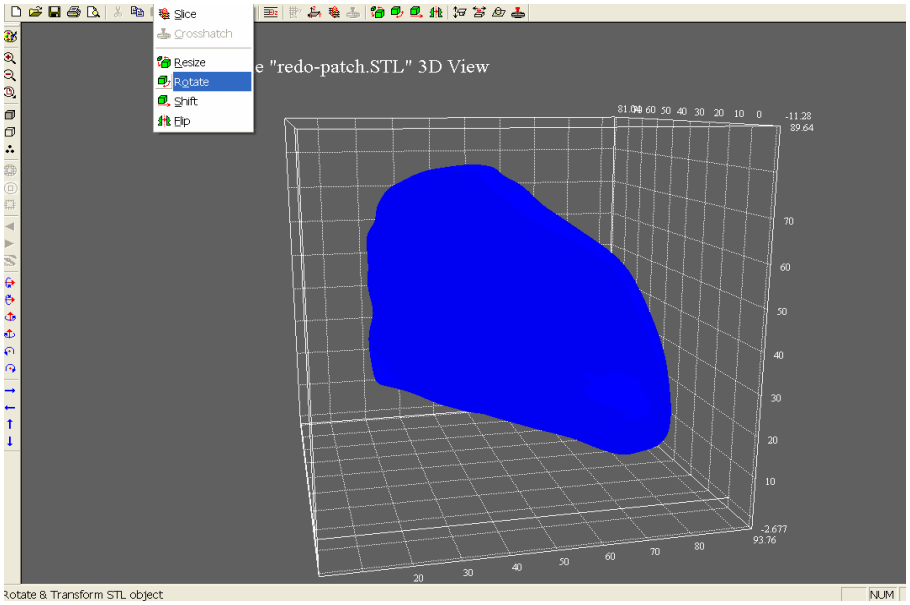
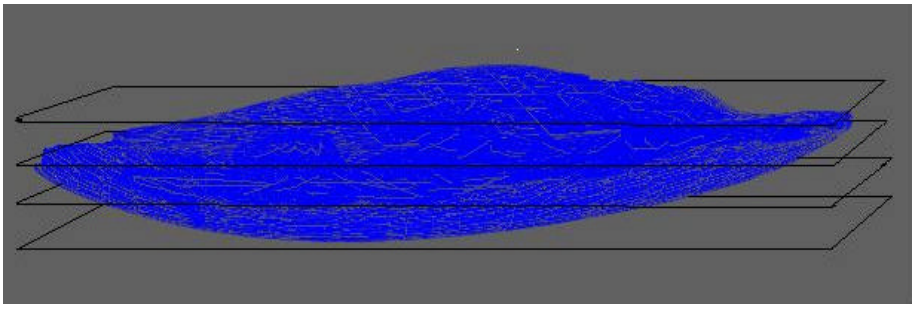
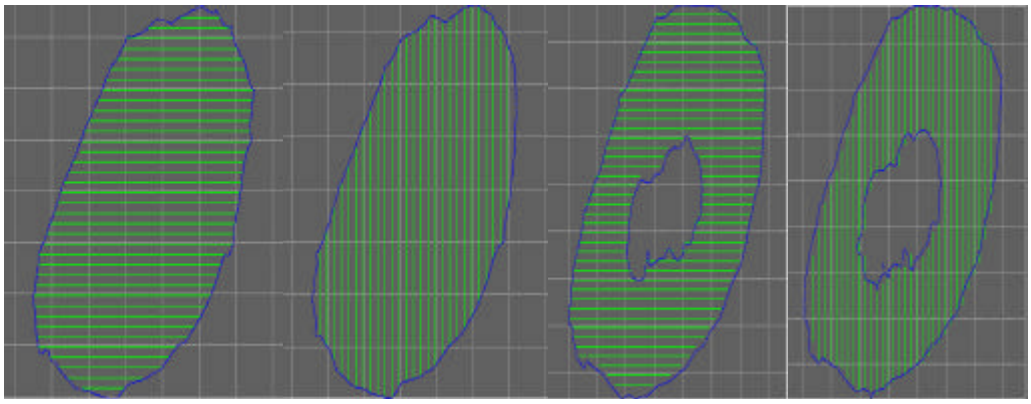


Figure 8 Model of skull defect patch shown on the RPBOD monitor



(a) Sliced model



(b) Consecutive layers

(c) Scaffold part built (15 layers)

Figure 9 Chitosan scaffold of the patch built by RPBOD system

The built part shown in Figure 9(c) is based on the model shown in Figure 9(a) and indicates the potential of the system to build free-form scaffold. Parameters, such as strand distance and layer height, have significant effect on the quality of the built part. Presently, hanging sections of the built part are not supported. Future function will consider providing appropriate support structures.

4. Results and Discussion

The pneumatic dispenser extrudes the viscous gel through a small diameter (0.1 ~ 0.2 mm) needle at a pressure from 2 to 4 bar, depending on the dispensing rate and the size of the needle. However, when the solution is of low viscosity, it flows in an uncontrollable way. Therefore the pneumatic dispenser is not suitable for the dispensing of low-viscosity solution, such as NaOH solution. On the other hand, the mechanical dispenser can achieve dispensing of low-viscosity fluids at low flow rate of 0.5 μl/sec.

Greater flexibility and advantage can be achieved with the method of dual dispensing with different dispensers to suit the nature of the fluid to be dispensed. In the case of single dispensing of one solution into a container of another solution, there is the problem of gradual lowering of concentration and agitation of the solution in the container. These problems are eliminated in the dual and sequential dispensing of the solutions. Additionally, improved adhesion is achieved. Moreover, the operating speed is also improved since agitation of the solution that is dispensed into is not a problem.

5. Conclusion

The RP robotic dispensing system (RPBOD), combining RP technology with tissue engineering, provides much potential for the design and desktop manufacturing of biomedical scaffolds. Rapid prototyping of scaffolds by the RPBOD is presented using a biocompatible chitosan gel for tissue engineering. During the scaffold fabrication, high temperature is not required and with the multiple-dispenser feature, it allows fabrication with materials or material additives, which otherwise decompose under heat, as well as the incorporation of proteins and living cells. The porosity of the resulting scaffolds can be controlled to facilitate good ventilation and cell growth. Important challenges for further research are the incorporation of growth factors as well as cells seeding into the 3D dispensing plotting materials. Improvements regarding the mechanical properties and the growth of cells are also necessary.

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