Biomaterials 30 (2009) 6221–6227

Contents lists available at ScienceDirect

Biomaterials

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

Human microvasculature fabrication using thermal inkjet printing technology

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ARTICLE INFO

Article history:
Received 26 May 2009
Accepted 28 July 2009
Available online 19 August 2009

Keywords:
Inkjet printing
Human microvascular endothelial cell
Fibrin
Microvasculature

ABSTRACT

The current tissue engineering paradigm is that successfully engineered thick tissues must include vasculature. As biological approaches alone, such as VEGF, have fallen short of their promises, one may look for an engineering approach to build microvasculature. Layer-by-layer approaches for customized fabrication of cell/scaffold constructs have shown some potential in building complex 3D structures. With the advent of cell printing, one may be able to build precise human microvasculature with suitable bio-ink. Human microvascular endothelial cells (HMVEC) and fibrin were studied as bio-ink for microvasculature construction. Endothelial cells are the only cells to compose the human capillaries and also form the entire inner lining of cardiovascular system. Fibrin has been already widely recognized as tissue engineering scaffold for vasculature and other cells, including skeleton/smooth muscle cells and chondrocytes. In our study, we precisely fabricated micron-sized fibrin channels using a drop-on-demand polymerization. This printing technique uses aqueous processes that have been shown to induce little, if any, damage to cells. When printing HMVEC cells in conjunction with the fibrin, we found the cells aligned themselves inside the channels and proliferated to form confluent linings. The 3D tubular structure was also found in the printed patterns. We conclude that a combined simultaneous cell and scaffold printing can promote HMVEC proliferation and microvasculature formation.

1. Introduction

The goal of tissue engineering is to solve the organ donor shortage by fabricating the replacement for the lost or damaged tissues and organs [1]. Recently, there are many successes achieved in tissue engineering. However, these successes are limited to relatively thin tissue structures, like skin and bladder [2,3]. These engineered tissues can be supported by the diffusion of nutrients from the host vasculature. However, when the thickness of the engineered tissue exceeds to 150–200 μm, it will surpass the oxygen diffusion limitation. Therefore, tissue engineers must create functional vasculatures into the engineered tissues to supply the cells with oxygen and nutrients, and to remove the waste products from the cells [4]. This is an unsolved issue in traditional tissue engineering [5]. This critical issue could be solved by cell printing technology, which is based on inkjet printing.

Inkjet printing is a non-contact printing technique. Inkjet printers have the ability to reproduce the data onto substrate with tiny ink drops by receiving data from computers [6]. Drop-on-demand means the ink drops are ejected only where and when they are required to create the images on the substrate. The inkjet printer has high operating frequency, high orifice density, integrated power, and interconnected electronics. In thermal inkjet printers, small air bubbles are created by heating and then collapse to provide the pressure pulse to eject a very tiny drop of ink out of the nozzle [6]. The current pulse lasts a few microseconds and raises the plate temperature as high as 300 °C [7]. Inkjet printing technology has already been widely used in electronics and micro-engineering industries for printing electronic materials and complex integrated circuits [8]. Recently, inkjet technology has also been successfully applied in biomedical field [9]. Although biological molecules and structures are usually assumed to be fragile and sensitive, DNA molecules have been directly printed onto glass slides using commercial available inkjet printers for high-density DNA microarray fabrication [10].

Our lab has successfully developed a novel inkjet printing application using the commercially available inkjet printers to print cells and biomaterials for 3D cellular scaffolds [11]. We showed that the standard HP and Canon desktop inkjet printers can be modified to perform cell printing. Organ printing, defined as computer-aided inkjet based tissue engineering, has the advantage to construct 3D structures with living biological elements. An important advantage of this process is the ability to simultaneously deposit living cells, nutrients, growth factors, therapeutic drugs along with biomaterial

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doi:10.1016/j.biomaterials.2009.07.056

Table 1
Selected fibrinogen and thrombin concentration tests for fibrin polymerization via printing. The best polymerization result via printing was with 60 mg/ml fibrinogen, 50 unit/ml thrombin, and 80 mM $CaCl_2$. Minimum deformations and diffusions of printed fibrin pattern were observed at these concentrations.

	Fibrinogen (mg/ml)	Thrombin (unit/ml)	$CaCl_2$ (mM)	Polymerization time (min)	Observation of printed fibrin scaffold
Concentration tested 1	30	20	40	N/A	N/A
Concentration tested 2	60	50	40	10	Broken and discontinuous
Concentration tested 3	**60**	**50**	**80**	**6**	**Continuous with minor deformations**
Concentration tested 4	60	100	80	6	Diffused
Concentration tested 5	60	200	160	5	Seriously diffused

scaffolds at the right time and location [12–14]. This technology can also be used for the microvasculature fabrication using appropriate biomaterials and cells.

Fibrin plays a significant role in natural wound healing. Fibrin gel has been widely used as sealant and adhesive during surgery. Fibrin glue is used as skin graft and tissue-engineered skin replacement [15]. Fibrin can be produced from the patients' own blood and used as an autologous scaffold for tissue engineering [16]. Fibrin is polymerized using fibrinogen and thrombin solutions at room temperature [17]. Fibrin gels might promote cell migration, proliferation, and matrix synthesis through the incorporation of the transforming growth factor β and platelet derived growth factors [18]. Fibrin has also been utilized in tissue engineering to engineer tissues with skeletal muscle cells [19], smooth muscle cells [20], and chondrocytes [21].

Endothelial cells form the inner lining of the whole cardiovascular system and have a remarkable capacity to adjust their number and arrangement to suit local requirements. Almost all tissues depend on a blood supply and the blood supply depends on endothelial cells. Endothelial cells are the only cells to form capillaries. They create an adaptable life-support system spreading into almost every region of the body. Endothelial cells extending and remodeling the network of blood vessels make it possible for tissue growth and repair (angiogenesis) [22].

In our study, a modified Hewlett–Packard Deskjet 500 thermal inkjet printer was used to simultaneously deposit human microvascular endothelial cells and fibrin to form the microvasculature. HP Deskjet 500 inkjet printer has a droplet volume of 130 pL. There are 50 firing nozzles on the printer head and the actual heating occurs in a 10 -μs pulse. The energy supplied during the printing process is transferred into kinetic energy and heating of the ink drop. Mathematical modeling studies indicated that the bulk drop temperature in the ink rises between 4 and 10 degrees above ambient during printing. This makes it possible for printing living systems [23]. It has been proved successful to print cell suspensions [24].

2. Materials and methods

2.1. Materials

Human microvascular endothelial cells (HMVEC) were kindly provided by Professor Peter I. Lelkes at Drexel University. MCDB 131 medium, fetal bovine serum, penicillin and streptomycin, sodium bicarbonate, L-glutamine, hydrocortisone, human recombinant epidermal growth factor, heparin, Dulbecco's phosphate buffered saline solution (DPBS), trypsin–EDTA, fibrinogen from bovine plasma, thrombin from bovine plasma were from Sigma Chemicals (St. Louis, MO, USA). Live/Dead Viability/Cytotoxicity Kit for mammalian cells was from Invitrogen (Carlsbad, CA, USA). IEC Centra-8R centrifuge was from International Equipment Company (Needham, MA, USA). Microprocessor controlled 280 series water bath was from Precision Scientific (Winchester, VA, USA). Glass microscope cover slips and 35 mm tissue culture Petri dishes were from Fisher Scientific (Pittsburgh, PA, USA). HP DeskJet 500 inkjet printer and HP 51626A black ink cartridges were from Hewlett–Packard Company (Palo Alto, CA, USA). Advanced Micro Osmometer (Model 3300) was from Advanced Instruments, Inc. (Norwood, MA, USA). Zeiss Axiovert S100 UV microscope and Zeiss LSM 510 laser scanning microscope were from Carl Zeiss (Minneapolis, MN, USA). Electromechanical testing system was from MTS System Corporation (Eden Prairie, MN, USA). Hummer 6.2 sputter coater was from Anatech Ltd (Hayward, CA, USA). Hitachi S4700N field emission scanning electron microscope was from Hitachi (Tokyo, Japan).

2.2. Thrombin and fibrinogen solution preparation

In order to assure prompt and optimum polymerization of fibrin gel after printing, we conducted fibrin gel polymerizations with various combinations of fibrinogen, thrombin, and Ca^{2+} concentrations (a few tests are listed in Table 1). The

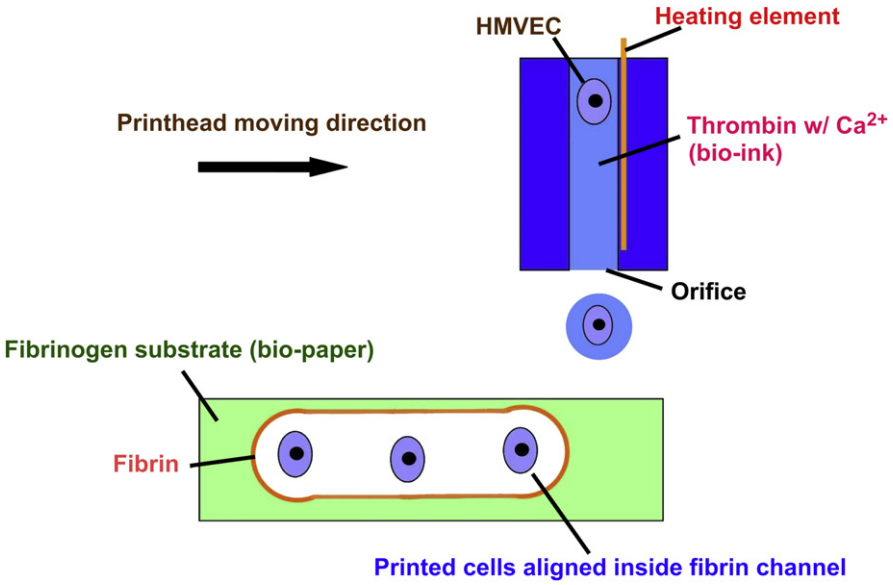

Fig. 1. Schematic drawing of postulated mechanism for simultaneous deposition of HMVEC and fibrin channel scaffold using modified thermal inkjet printer. When bio-ink is printed into fibrinogen substrate to form fibrin channel, the cells in the bio-ink are deposited into the scaffold at the same time as the fibrin channel fabrication. The printed cells are aligned inside the fibrin channels and ready for proliferation.