SLS Fabrication of Highly Porous Model Including Fine Flow Channel Network Aiming at Regeneration of Highly Metabolic Organs

Toshiki NIINO, Yasuyuki SAKAI, Hongyun HUANG and Hiromichi NARUKE
The Institute of Industrial Science, The University of Tokyo, JAPAN
4-6-1 Komaba Meguro Tokyo, 153-8505 Japan
niino@ieee.org

Reviewed, accepted September 14, 2006

ABSTRACT

Fabrication of very porous scaffold for regeneration of highly metabolic organs is reported. Polycaprolactone (PCL) powder was mixed with fine salt grains as filler and SLS processed to develop a model including fine flow channel network. The fabricated model was rinsed with water to dissolve the salt, and high porosity of 90% was successfully obtained. Additionally, residual powder in the channels was effectively removed by solution of the filler. Through micro-CT observation, it was confirmed that channels of which diameter was smaller than 1mm were successfully fabricated and repeating branching and merging. Result of culture test is also reported.

INTRODUCTION

Many diseases and accidents can damage organs and/or tissues irreparably. In these cases, artificial organs or whole organ transplantation is the therapy of choice. Many artificial organs such as contact lens and pace makers are quite successful, but their successes are quite limited by low biocompatibility and their limited function(s). Reducing immune rejection problem recent developments in pharmacology have enabled organ transplantation from others. However, finding an exact donor is still very difficult. Contrarily, regenerative medicine can avoid these problems since it uses patients' own stem cells. There are a lot of clinical successes for regeneration of two dimensional tissues such as skins. And now many research are concentrated on regeneration of three dimensional tissues or organs, we need three dimensional scaffolds.

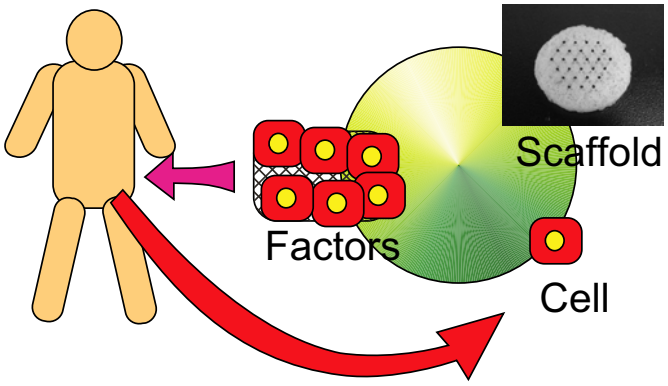


Fig. 1 Three Elements of Regenerative Medicine

Basically, tissue or organ regeneration technology is

composed of three elements of cell, scaffold and culturing factor. Among these elements, a scaffold is employed to define geometry of tissues or organs to be regenerated, as its primitive role, by guiding the position of cultured cells. For this role, scaffold is required to be highly pore and biodegradable for the most cases. To fabricate such scaffolds, several methods including solvent casting and particulate leaching [1], gas foaming [2], fiber bonding [3], phase separation [4], melt molding [5], emulsion freeze drying [6], solution casting and freeze drying [7] were reported. In these literatures, discussion is focused on fabrication of porous biomaterial but giving them three-dimensional geometry. For that purpose, application of solid freeform fabrication technologies is quite advantageous, and many efforts have been focused on research to develop three-dimensional scaffolds as reviewed in literature [8]. Moreover, SFF technologies have potential of controlling pore size, pore geometry, pore interconnectivity and spatial distribution of pores. With respect to advantage of using SFF technology in scaffold fabrication, several authors have reviewed in literature [8][13]. Taking advantages of SFF, several successes in bone regeneration of animals have been reported [14] [15] showing that the same remedy becomes technically possible to human in the near future. There are a few tissues or organs of which cells can survive and proliferate without mass transportation of nutrition and oxygen by capillary vessels located very close to the cells themselves, and bone and skin are some of such rare cases. Contrarily, in the most cases of internal tissue or organs, cells must be located in the range of 3mm, at the most, from supporting vessels, or their necrosis will occurs. Among these organs, the liver is currently a suitable target due to its characteristic function and structure. Although the liver has a very complex and highly organized structure, the first step in the engineering is limited to two main stages: obtaining a large number of progenitor liver cells, and arranging them in a three-dimensional scaffold such that the unit-volume-based functions are enhanced to levels nearly comparable to those in vivo. This situation is likely to be better than other organs such as the lung or the kidney. For examples, in the case of lung tissue engineering mechanical flexibility should be possessed in addition to biological functions, and in the case of kidney tissue engineering, different roles should be played in the more complicated hierarchical structure.

With respect to liver regeneration, approach of Vacanti's group is very famous [16], [17]. They are trying to complete regeneration of liver with the same dimension of human liver including capillary vessels of which diameter is around 10μm at the minimum. However, currently, no three-dimensional fabrication methods that can generate such a large dimension of object with such a precise resolution have been established. As for

resolution, only, we can find several methods such as IC fabrication technique, but unfortunately, all of them are two dimensional. Hence, there are several approaches in which two-dimensional sheets including very fine channel network is fabricated and laminated manually afterward [18][20]. However, at this moment, these approaches take too much manual assembly cost to stack the sheets, of which thickness is almost the same as resolution in its planar directions, up to final liver's height. The authors of this paper take another approach. In their approach, scaffold is fabricated by commercially available SFF system or its slightly modified version. In each scaffold, flow channels are constructed, but their precision is not as much as natural liver's because of insufficient parts building resolution of the SFF systems. To the cell away from the channels, oxygen is supplied through diffusion in the culture medium. According to Sakai's estimation, the maximum rage from a flow channel in which liver cell can be provided with oxygen through diffusion is less than 200μm if the scaffold is filled with cells at the same density as human liver of 2.5×10^8 cells/cm^3 [21]. Since the distance between two channels can be roughly calculated as the double of the maximum distance from a cell to a channel, distance between neighboring channels must be smaller than 400μm. To reserve room for cell culture, we should put some restriction on the diameter of flow channel and provide good flow channel design. Otherwise, scaffold would be filled with flow channel but cells when cell culture would be successfully performed. Thus, diameter of channel should be 100μm. For currently available SFF systems, constructing channels with this thinness is still very difficult. However, while cell density is lower than the value mentioned above, required scale of flow channel increases in inverse proportion of the density. Until the pores are completely filled with cell, we can expect flow of culture medium through the pores. Furthermore, we might reconstruct such microstructure with help of high angiogenesis ability of liver cell or liver stem cells [22]. Accordingly, cell culture is performed *in-vitro* with solid freeform fabricated scaffold, of which precision is not sufficient for complete regeneration, up to a certain intermediate cell density, and following *in-vivo* culture will reconstruct microstructure with help of angiogenesis ability of liver cells in the approach of the authors. Following this approach, Sakai, a co-author of this paper, developed such a scaffold by manually stacking highly porous plates that are made of biodegradable plastic and pierced by drilling [21]. This literature reported experimental results that support requirement for fine flow channels.

Up to now, various scaffolds have been fabricated with a variety of SFF techniques. Among them, fabrication utilizing extrusion of liquid or liquidified material and solidification such as FDM is quite reasonable because of its high resolution and ability

in controlling pore size and density [23][26]. In addition, it is possible to seed into the material in some systems which do not utilize heat to liquidify their material. Since filling rate, subtraction of porosity from 100%, and pore size are dominated by rate of raster gap against raster interval and raster gap, respectively, requirement for high porosity and small pore size leads to requirement for very fine raster interval and fine extrusion nozzle. For example, to obtain pore size and porosity of 100μm and 90%, which are required in this research, we have to extrude very fine filament of 10μm. This might be possible, but reliability in building large scaffold seems relatively low.

In terms of part building resolution, SLA is the highest among all the SFF fabrication methods, and several applications on scaffold fabrication have been reported [27], [28]. However, it is reported that removing highly viscous uncured resin from all the pores and flow channels of large scale scaffold is quite difficult.

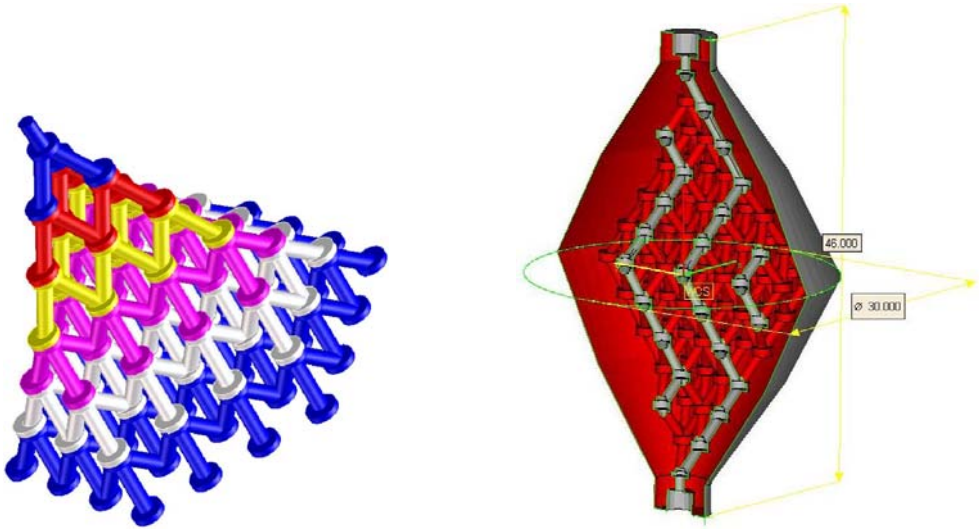
Kim et al. fabricated a scaffold that included interconnected longitudinal and radial cannels with a 3DP system [29]. In this fabrication process, leachable salts and solvents were used to give very high porosity to their scaffold. However, use of solvent should be avoided because of toxicity of residual.

SLS technique has also been utilized in fabrication of scaffolds [14][30]-[31]. It seems possible that high porosity around 90% can be obtained by SLS process incorporated with porogen like 3DP [29] without using toxic solvent. However, fabrication of scaffold with high porosity around 90% has not been reported.

This paper describes application of laser sintering technology on fabrication of scaffolds including very thin flow channel network. In order to obtain high porosity, sodium chloride is used as porogen. Obtained scaffold is observed by a micro X-ray CT system. Amount of residual porogen is estimated. Culture test of liver cells is also performed.

FABRICATION METHOD OF HIGHLY PORE BODY BY SLS PROCESS

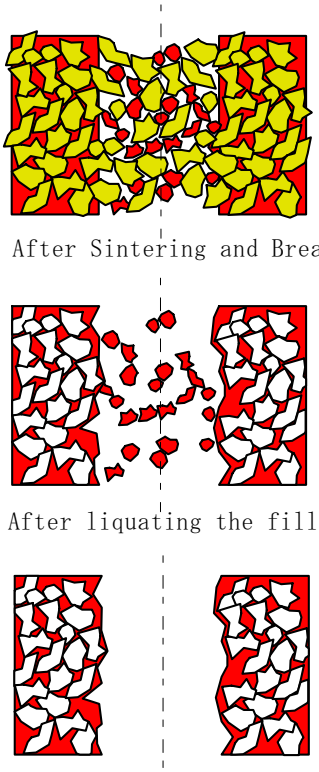
Fig. 2 represents the design of the scaffold based on the fundamental concept described above. Fig. 2a depicts upper half of the flow channel network that is included in the scaffold. The flow channel network is composed of accumulated flow channel running along slant edges of a tetrahedron. Such tetrahedrons are accumulated in 6 layers to organize a flow cannel network. This network is placed in a spindle shape and very porous sponge created by selective laser sintering process and following post process described below.



(a) (b)

Fig. 2 Schematic views of scaffold. (a) Basic 3D structure of the upper half of flow channel network. (b) Schematic partially cross-sectional view of the scaffold.

It is difficult or almost impossible to create completely dense object by powder based solid freeform fabrication systems. This difficulty or impossibility imposes limitation on freedom of selecting material without degrading physical strength of created parts or object in industrial applications. This disadvantage seems to become advantage in fabrication of porous scaffold, but still unfortunately, required porosity, that is around 90% in regeneration of liver, is not obtained since porosity of original powder or so-called "cake" is lower than 70% and lowered after being sintered. To obtain such a high porosity, plastic powder is blended with water-soluble salt and SLS processed, and the salt is leached afterward. In addition to porosity improvement, use of the salt brings other advantages. For the first, the salt plays an important role in reduction of curl of a part since the melt temperature of salt is much higher than those of the most plastics. Accordingly, we can expose stronger laser power to improve strength of the very porous scaffold. For the second advantage,



(a) After Sintering and Breaking

(b) After liquating the filler out

(c) Removal of not sintered powder

Fig. 3 Removal of not sintered powder from flow channel

disappearance of the salt improves removability of not sintered powder. It is easy to imagine that removing not sintered powder from very complicated and thin flow channel as shown in Fig. 2, is very difficult or impossible. However, leaching process dissolves the salt that occupying most volume of the total powder and removes the not sintered powder as shown in Fig. 3.

EXPERIMENTS

Materials

Polycaprolactone (PCL) was used as biodegradable material of the scaffold. This PCL is provided by Japanese company, Daicel Chemical Industry Ltd., under trade name of "Praccel H5." This plastic's molecular weight, melting temperature, degradation temperature and density are 50,000, 60°C, 230°C and 1.14g/cm^3, respectively. By making emulsion of PCL in PEG, the plastic was formed into spheres with diameter of 50μm on average. As porogen or filler, sodium chloride that is sieved into the range between 100μm and 150μm are blended with PCL at a rate of 4:1 in weight.

Apparatus and Process Parameters

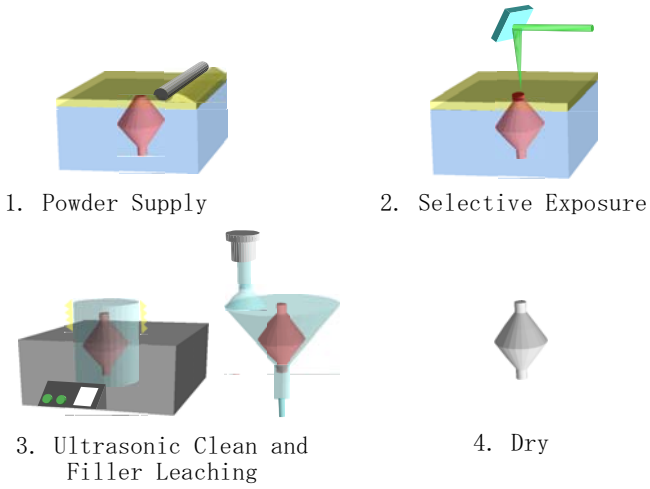


Fig. 4 Process For Fabricate Porous Object

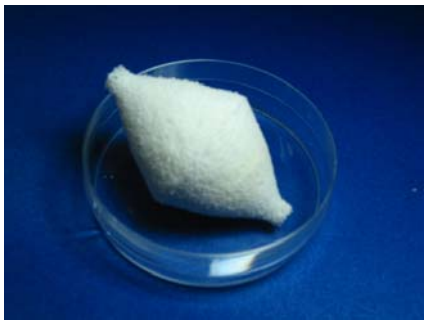


Fig. 5 Photo for Scaffold

Fig. 4 depicts fabrication process of highly porous body. Laser sintering was performed by CO_2 laser focused on the powder surface into spot diameter of 500μm. Temperature of the powder surface was controlled at 50°C. Preliminary tests for building a thin straight and vertical hole were performed to find adequate laser power, scan pitch and scan speed. As a result, 7.8W, 0.10mm and 3433mm/s were selected for laser power, scan pitch and scan speed, respectively, since these parameters brought the minimum diameter of 0.5mm for a straight and vertical hole with a length of 3mm. SLS processed object was roughly rinsed and put under flowing water for two hours. After this,

ultrasonic cleaning was performed and rinsed again with flowing water for 10hours to remove the salt and not sintered plastic powder.

RESULTS

Fig. 5 is photo for developed scaffold. That was 46mm and 30mm in length and diameter at the middle, respectively. Its volume and weight are 13cm^3 and 1.7g, respectively, and porosity was calculated as 89%. In the scaffold, flow channel network is composed by accumulating tetrahedrons, each of which has 4-mm-long edges. Since the spot size of employed laser is not small enough to develop desired diameter of flow channel, magnified design was used. Thicknesses of the flow channels were designed to be 1.0mm and 2.0mm at the edge and the junction, respectively, while desirable thickness of flow channel is 100μm.

X-ray CT observation was performed using SMX-225CT fabricated by SHIMADZU Corporation. Fig. 6 and 7 show X-ray image and CT reconstructions, respectively. To obtain the original X-ray image with high contrast the scaffold is soaked with iohexol 300mg/mℓ (Omnipaque300: Daiichi Pharmaceutical Co., Ltd.). In the reconstructed cross sectional pictures, no stuffing channel was found to verify if powder was successfully removed from the complicated flow channel network. Diameter at the edge and junction is 0.9mm and 1.7mm on average, respectively.

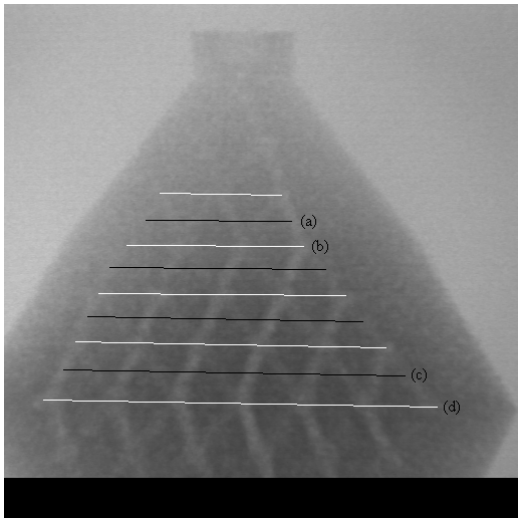


Fig. 6 X-ray photo for a scaffold

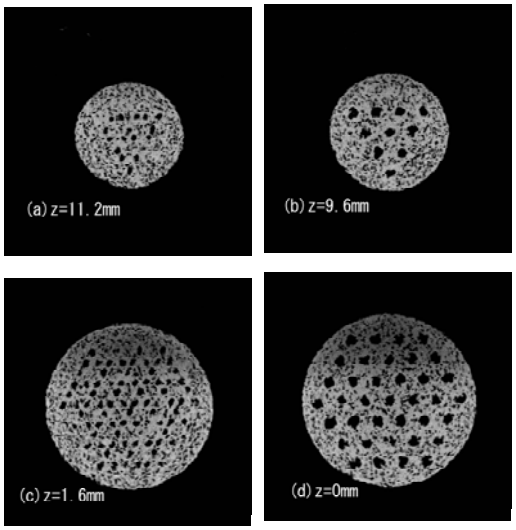


Fig.7 CT reconstructed cross section

To confirm complete removal of sodium chloride, a scaffold before rinse is soaked in 200cm^3 of water and concentration of sodium chloride was measured every two hours. The water is refreshed after every measurement, and the concentration lowered as shown Tbl. I. After 8 hours, the Concentration reached 5.1x10^{-5}mol/ℓ where that for physiological salt solution is 1.54mol/ ℓ. These results indicate that approximately 10

Tbl. I Variation of Salt Concentration

Rinse Time [hours]	2	4	6	8
Salt Concentrati [mol/ℓ]	$5.5x10^{-1}$	$6.6x10^{-4}$	$1.1x10^{-4}$	$5.1x10^{-5}$

Tbl. II Cell Growth for Various Scaffolds. Perfusion culture was performed for 11days and 14days using SLS processed scaffolds and drilled one, respectively.

	Number of Cells [million		Cell Growth [times]	Cell Density
	First Day	Day 11/14		
SLS with channel	53.0	99.4	1.9	7.6
SLS without channe	58.0	53.5	0.9	4.1
Drilled channel	6.0	9.6	1.6	7.2

hours rinse with water can reduce the salt enough for cell culture.

To evaluate the effectiveness of the scaffold, several perfusion culture tests were performed. In the tests, human hepatoma Hep G2 cells were used as seed. Tbl. II compares cell growth after culture for various scaffolds. Flow channel equipped and SLS processed scaffold showed much better performance than that without flow channels. Between SLS processed scaffold and drilled scaffold, small but significant difference was observed. On day 11, 6.3μg/million cells/day of albumin production rate was obtained. This value was more than 6 times of previously tested drilled scaffold and reached 20% of human liver performance. Final cell density in case of SLS scaffold with flow channel, of $7.6x10^6/cm^3$, is equivalent to 3% of human liver.

DISCUSSION

In this research, SLS fabrication of a scaffold with a fine flow channel network is tried. Precision of the obtained network represented by channel diameter of 1mm is insufficient to culture the cells to the same density as human liver, but it is reaching the highest standard of commercially available SLS machine. Literature [32] reported that resolution of SLS can be improved by using precisely focused laser and fine powder. This strongly supports the possibility of improving precision of scaffold. Above all, it is remarkable that such complicated geometry of the flow channel is created and not sintered powder is removed from the channel completely; there is a report of narrower straight holes [33] but bending or zigzag ones as fabricated in this research. In this research, advantage of using water-soluble filler in fabrication of scaffolds for highly metabolic organ regeneration is clearly indicated, but effect of varying parameters relating to the filler such as grain size and blend rate is still unclear. Optimization of these parameters should be performed taking care of perfusion tests in the near future.

CONCLUSIONS

SLS fabrication of porous PCL scaffold was tested. To obtain very high porosity, granule of sodium chloride was blended to the plastic powder. A scaffold including flow channel network is designed and successfully fabricated without curl. Not sintered powder in the flow channels were completely removed owing to dissolution of the porogen. High porosity of 89% was obtained. X-ray CT observation confirmed that no channel was stuffed and diameter of the channel was as small as being designed. The porogen was removed perfectly for following cell culture. Perfusion culture tests were performed and good cell growth and albumin production were observed. These results support good possibility of using SLS technique with water-leachable filler in fabrication of scaffold for highly metabolic organs. Thick flow channels in this report were not desirable, but the thickness seems able to be reduced by using thinner laser beam.

References

[1] Mikos, A. G. *et al*. Preparation and characterization of poly (L-lactic acid) foam. *Polymer* 35, 1068-1077

[2] Mooney, D. J. *et al*. Novel approach to fabricate porous sponge of poly (D, L-lactic-co-glycolic acid) without the use of organic solvent. *Biomaterials* 17, 1417-1422

[3] Freed, L. E. *et al*. Biodegradable polymer scaffolds for tissue engineering. *Biotechnology* 12, 689-693

[4] Lo, H. *et al*. Fabrication of controlled release biodegradable foams by phase separation. *Tissue Eng.* 1, 15-28

[5] Thomas, J. I. *et al*. Fabrication of biodegradable polymer scaffolds to engineering trabecular bone. *J. Biomater. Sci.Polym. Ed.* 7, 23-28

[6] Wang, K. et al. A novel method to fabricate bioabsorbable scaffolds. *Polym.* 36, 837-842

[7] Hsu, Y.Y. *et al.* Effect of polymer foam morphology and density on kinetics of *in vitro* controlled release of ionized from compressed foam matrices. *J. Biomed Mater Sci* 35, 107-116

[8] Yeong, W. Y. *et al*: Rapid prototyping in tissue engineering: challenges and potential ; *TRENDS in Biotechnology*, 22, 643-652

[9] Hutmacher, DW. Scaffolds in tissue engineering bone and cartilage, *Biomaterials* 21, 2259-2543

[10] Landers, R. *et al*. Fabrication of soft tissue engineering scaffolds by means of rapid

prototyping techniques. *J Mater Sci*, 37, 3107-16

[11] Sachlos, E. *et al*. Making tissue engineering scaffolds work. Review on the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. *Euro Cells Matter*, 5, 29-40

[12] Sun, W. *et al*. Computer aided tissue engineering part I: overview, scope, challenges. *J Biotech Appl Biochem*, 39, 29-47

[13] Sun, W. Recent development on computer aided tissue engineering -a review. *Comp Method Prog Biomed*, 67, 85-103

[14] Das, S. *et al*; Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials*, 26, 4817-4827

[15] http://www.vm.a.u-tokyo.ac.jp/geka/research/reconst/clinical%20data/index.html (in Japanese)

[16] Kaihara, S. *et al*. Silicon micromachining to tissue engineer branched vascular channels for liver fabrication. *Tissue Eng*, 6, 105-117

[17] Vacanti, JP. *et al*. Translating technologies to build whole vital organs. *Tissue Eng* 8 1114-1115.

[18] Borestein, *et al*. Microfabrication technology for vascularized tissue engineering. *Biomed Microdevices* 4, 167-175

[19] Leclerc, E. *et al.* Cell culture in a three-dimensional network of PDMS microchannel, *Biomed Microdevices* 5, 109-114

[20] Leclerc, E. *et al*, Fabrication of microstructures in photosensitive biodegradable polymers for tissue engineering applications, *Biomaterials* 25, 4683-4690

[21] Sakai, Y. *et al*: A novel poly-L-lactic acid scaffold that possesses a macroporous structure and a branching/joining three-dimensional flow channel network: its fabrication and application to perfusion culture of human hepatoma Hep G2 cells ; *Mat Sci and Eng* C 24 379–386

[22] Suzuki, A. *et al*. Clonal identification and characterization of self-renewing pluripotent stem cells in the developing liver, *J Cell Biology* 156, 173-184

[23] Too, M. H. *et al*. Investigation of 3D non-random porous structures by fused deposition modeling. *Int J Adv Manuf Technol* 19, 217-223

[24] Zein, I. *et al*. Fused deposition modeling of novel scaffold architectures for tissue engineering applications. *Biomaterials* 23, 1169-1185

[25] Chen, Z. *et al*. Fabrication of osteo-structure analogous scaffolds via fused deposition modeling, *Scripta Materialia*, 52, 157-161

[26] Wang, F. *et al*. Precision extruding deposition and characterization of cellular poly-ε-caprolactone tissue scaffolds. Solid Freeform Fabrication Symposium,

Proceedings 2003, 573-584

[27] Matsuda, T. Liquid acrylate-endcapped biodegradable poly(-caprolactone-co-trimethylene carbonate). II. Computer-aided stereolithographic microarchitectural surface photoconstruct. *J Biomed Mat Res* 62, 395-403

[28] Sakai, Y. Ultraviolet-irradiation-based photofabrication that simultaneously produces a macroporous structure and flow channels using a photoreactive biodegradable polymer and a gas-forming azoamide compound. *Biochem Eng J*, 20, 163-171

[29] Kim, SS. *et al.* Survival and function of hepatocytes on a novel three-dimensional synthetic biodegradable polymer scaffold with an intrinsic network of channels. *Ann Surg* 228, 8-13

[30] Tan, K. H. *et al*. selective laser sintering of biocompatible polymers for applications in tissue engineering, *Bio-Med Mat and Eng*, 15, 113-124

[31] Popov, E. N. Selective laser sintering of 3-D biodegradable scaffolds for tissue engineering, Mat. Res. Soc. Symp. Proc. Vol. EXS-1, 51-54

[32] Regenfuss, P. Industrial freeform generation of microtools by laser micro sintering. Solid Freeform Fabrication Symposium, Proceedings 2004, 709-719

[33] Das, S. *et al.* Freeform fabrication of nylon-6 tissue engineering scaffolds. *Rapid Prototyping J* 9, 43-49