Pyrex Journal of Medicine and Medical Sciences

Vol 4 (3) pp. 11-17 May, 2017

http://www.pyrexjournals.org/pjmms

Review paper

3D Bio-Printing: A Promising Technique to Fabricate Dermal Equivalent for Skin Wound Healing

Juan Du[1] and Xuelai Liu[2*]

[1]Department of Endocrinology, The People's Hospital of Jilin Province, Changchun, China.
[2]Department of Surgery, Li Ka Shing faculty of medicine, The University of Hong Kong, Hong Kong, China.

Accepted 6 May, 2017

Abstract

The wound especially the skin wound healing is the commonest problem faced by surgeons. Several technologies have become available for skin tissue engineering that adopts a fundamentally different and novel approach recently. Among these, the applications of 3D bio-printing opened a new way to heal wound. There were four parts in this review. First, we introduced the concept of 3D bio-printing briefly. The main 3D bio-printing technique included ink-jet printers and Laser associated 3D printer. Second, we summarized systematically extra cellular matrix being the basic scaffold of 3D bio-printing. Thirdly, the application of 3D bio-printing in both *in vitro* and *in vivo* artificial skin tissue was introduced. At last, we outlook the future of application of 3D bio-printing in wound healing.

Keywords: 3D bio-printing, skin, wound healing.

INTRODUCTION

The skin is the largest organ of the human and animal body. It plays a critical role in maintaining homeostasis and providing protection from the external environment. The highly complex and multi-layer structure of the skin provides a physical barrier to prevent the entry of xenobiotics into the body, and regulates the transport of water or small metabolites outside of body, both the acute wounds originating from physical or chemical trauma and chronic wounds including tumor skin ulcer, vascular ulcer or diabetic foot ulcer, can significantly destroy skin barrier and impair its physiological functions. For example, in which a considerable amount of skin has been lost by injuries, it becomes critical to replace the impaired skin via grafts to protect water loss from the body, as well as to mitigate the risk posed by opportunistic pathogens, Lee *et al.,* 2014.Skin grafts can also promote the wound healing process greatly and restore the barrier and regulatory functions potentially at the site of the wound. So during the time to treat the cutaneous defects, the application of artificial dermal substitutes is accepted increasingly and widely approach for skin transplanting, especially being one of the most effective biological tool after acute thermal trauma, Hodgkinson and Bayat, 2011.

3D (3 dimensional) bio-printing, a flexible automated on-demand platform for the free-form fabrication of complex living architectures, is a novel approach for the design and engineering of human organs and tissues, for instance the skin, cartilage, bone, tendon and cardiac tissue, Zhang *et al.,* 2016; Futrega *et al.,* 2015; Shamaz *et al.,* 2015; lee *et al.,* 2015; Thankam and Muthu, 2015. Among these, 3D bio-printing offers significantly advantages compared with classical skin tissue engineering, which presents tremendous potential in the

*Corresponding author email: liuxuelai_steven@163.com

fabrication of 3D skin tissue. The principle of 3D bio-printing is to use 3D computer models for the reconstruction of a 3D physical model by the addition of biomaterial layers. In fact, 3D bio-printing is a method using 3 dimensional computer-aided design and layer-by-layer build-up of the biomaterials for producing 3D tissues or organs. It is also referred to as rapid prototyping which deposits drops or fibers of cell-laden hydrogels on a platform that consists of multiple cell types and matrix at predefined locations within a porous 3D structure. The great advantage of 3D bio-printing is its ability to create almost any complex shape or geometric feature, Rengier *et al.,* 2010; Georgios *et al.,* 2012. This process produces bio-engineered structures that can be applied in tissue engineering, regenerative medicine, pharmacology and basic cell biology study, Guillotin and Guillemot, 2011. It also has a broad range of applications in transdermal and topical formulation discovery, dermal toxicity studies, and in designing autologous grafts for wound healing.

In this paper the current state of knowledge in the field of 3D bio-printing for skin tissue has been reviewed. For this purpose it is introduced the concept of 3D bio-printing and presented the application of 3D bio-printing.

BRIEF INTRODUCTION OF 3D BIO-PRINTING

In general, the 3D bio-printing approaches can be divided into two groups: orifice and orifice-free techniques. In the field of orifice techniques, modified ink-jet printers are commonly used for printing of biological materials through a small nozzle, Figure 1. There are thermal, piezoelectric, and electromagnetic approaches to create drops on demand, Xiaofeng *et al.,* 2012.Ink-jet droplets form hydrogel building blocks at the landing position. The kinetics of gelation and sedimentation of droplets can be adjusted to the ejection rate of the printed droplets to fabricate a tubular structure ‘on-the-fly’. Similarly, a cell-laden fibrin pattern has been printed by depositing droplets of cell-containing fibrinogen solution into a thrombin solution, Guillotin and Guillemot, 2011. Some biomaterials including E. coli, DNA arrays, protein patterns, and living cells have been printed by this technique successfully. But ink-jet printers are capable to handle cell suspensions only with low viscosities and low cell densities to avoid shear stress at the orifice or cell clogging, Koch *et al.,* 2012.

An orifice-free technique, which is Laser bio-printing, can print hydrogel precursors with any desired viscosity and any desired cell density. It has been implemented into different technologies named direct write printing, Guillotin and Guillemot, 2011. The physical principle of laser bio-printing is based on the generation of a cavitation-like bubble, into the depth of the bio-ink film, whose expansion and collapse induces the formation of a jet and, thereby, the transfer of the bio-ink from the ribbon to the substrate, Fig. 2.

Briefly, the experimental setup consists of two coplanar glass slides. The upper one is covered underneath with a laser absorbing layer and layer of the cells containing material to be transferred. Laser pulses are focused through the glass slide into the absorption layer which is evaporated locally. This generates a high pressure that propels the subjacent cell compound towards the lower glass slide. A material sheet, scaffold or a layer of gel can be positioned on the lower glass slide to print cells onto or into it, Koch *et al.,* 2012.

To print cells with high resolution and high throughput, parameters related to laser pulse characteristics (such as wavelength, pulse duration, repetition rate, energy and beam focus diameter), bio-ink properties (such as viscosity, thickness and surface tension) and substrate characteristics should be adjusted. Previous studies showed that laser bio-printing could print mammalian cells without affecting viability and function, and without causing DNA damage, Guillotin and Guillemot, 2011. The differentiation, proliferation, heat shock protein and the immunephenotype are not different between printed cells and control cells. These studies indicate consistently that cells are not affected by laser printing, Koch *et al.,* 2013.

Above all, the 3D bio-printing technique is a complicated, multi-variable process and is affected by properties of both biomaterial and printing device. The size of the biomaterial is determined by the interaction of a number of factors, including dispensing orifice dimension, solvent, fluid viscosity, surface tension, fluid-surface interactions, polymer concentration, humidity, and temperature. To complicate the printing process, many of these variables are influenced by each other, Chang *et al.,* 2011.

A lot of environmental factors and equipment characteristics should be considered for fabricating tissue successfully. A major concern is the maintenance of a sterile work area and preventing sample contamination while printing. The small bio-printers may permit operation within a biosafety hood. Larger commercial systems offer the option of environmental isolation and permit the addition of filters and laminar flow capabilities to minimize work area contamination, Chang *et al.,* 2011. Bio-printer components, particularly instrumentation in contacting with biomaterials directly, should be designed for compatibility with standard sterilization techniques.

EXTRA CELLULAR MATRIX IS THE BASIC SCAFFOLD OF 3D BIO-PRINTING

Biological tissues are composed of different type cells that embedded in their specific extra cellular matrix (ECM), with interwoven vasculature. Most mechanisms of pattern formation are based on spatial and temporal

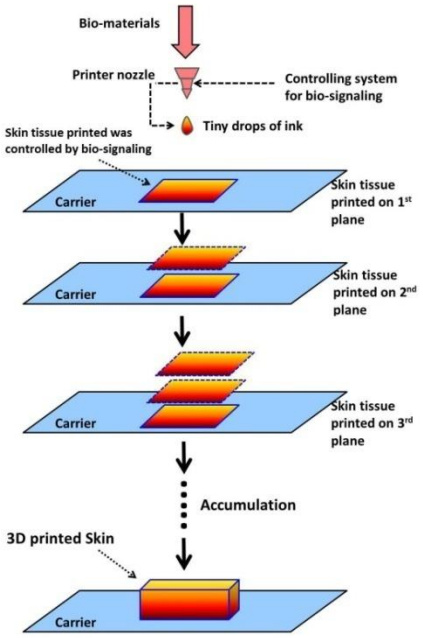

Figure 1. The mimetic diagram of 3D bio-printing technique

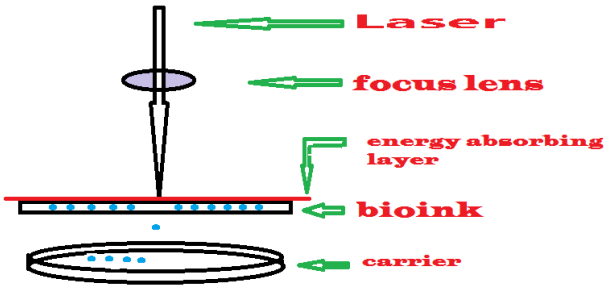

Figure 2. The model of laser associated bio-printing

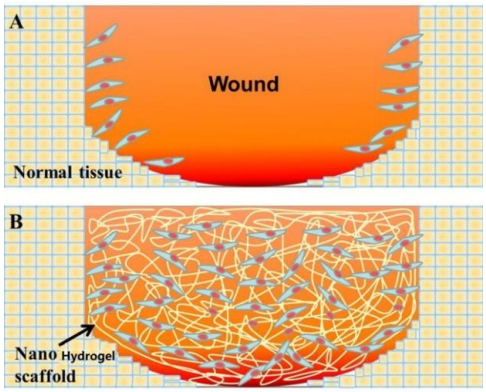

Figure 3. The mimetic diagram of nano fibrous scaffold filled in injury skin tissue can be fabricated via 3D printing technique. Keratinocytes and fibroblasts are respectively distributed on surface of skin. Once sever damaged, they are difficult to regenerate or regenerate slowly in situ due to deprivation of extracellular matrix and adhesion environment. The scaffold filled in damaged skin wound can provide or supply above deprivation, through which the regeneration of functional cells or tissue are enhanced. Currently, this nano fibrous scaffolted as a biological module, can be easily fabricated via 3D printing technique.

heterogeneity during tissue development and remodeling. These induces the formation of a local microenvironment, including gradients of soluble or insoluble factors, and physical forces, Guillotin and Guillemot, 2011. It is important to control the interactions between cells and microenvironment in tissue engineered and regeneration medicine that mimic native tissue architecture and for guiding cellular differentiation and organization, Mariah *et al.,* 2006.

The 3D bio-printing need a scaffold of the desired shape and internal structure with incorporation of multiple living cells that can form the tissue once implanted. As the natural scaffold, existing ECM is still extensively used. That includes protein (e.g. collagen, fibrin) and polysaccharide-based materials (e.g. chitosan, alginate, glycosaminoglycans, and hyaluronic acid), Berthiaume *et al.,* 2011.

An ideal scaffold should have the following main characteristics: (1) Being bio-compatible and bio-resorbable so that can be controlled degradation and resorption rate to match cell/tissue growth in vitro and/or in vivo; (2) Having a suitable chemical surface for cell attachment, proliferation and differentiation; (3) Being 3 dimensional and an interconnected highly porous network for cell growth, flow transport of nutrients and metabolic waste;(4) Being fabricated in a variety of shapes and sizes easily; (5) Inducing no adverse immunological response either directly or the degradation byproducts, Guillotin and Guillemot, 2011.

Many proposed tissue strategies currently are based on the use of hydrogels and porous scaffolds. Hydrogels are popular materials to be used in bio-printing as scaffold because they have high water content, excellent bio-compatibility, tunable mechanical properties, permeability to nutrients and biodegradability. Lots of hydrogels can be gelled under mild, cytocompatible conditions. Because of providing a suitable environment for cell survival and differentiation, the development of hydrogels with an optimized combination of process ability for use in 3D bio-printing techniques and cytocompatibility is an essential step towards the future application of organ printing, Fedorovich *et al.,* 2011. The majority of the hydrogels used for organ printing provide the cells with a non-interactive encapsulation matrix, which mainly acts as a template to permit cell localization, figure 3.

Cell adhesion is mediated by the interaction of surface receptors with ligands bound to ECM proteins. For mimicking that process, polyethylene glycol (PEG) hydrogels conjugated with integrin-binding receptor peptide increased dramatically adhesion of fibroblasts and endothelial cells. The discovery that PEG does not elicit an immune response was a significant milestone, leading to its wide spread use in tissue engineering as an inert matrix to study the interaction between cell and biomaterials. A variety of biomolecules including short peptides, proteins, polysaccharides, antibodies, and DNA molecules have been conjugated to hydrogels for controlling cell survival, adhesion, matrix degradation and organization, migration, differentiation and apoptosis. The results of these studies demonstrated clearly that bio-molecules maintain their activity after conjugation to an otherwise inert hydrogel network, Jabbari, 2011. These scaffolds can be seeded heterogeneously with different cells by laser printing.

Self-assembly as a natural attempt of lowering system energy and usually the most common interactions between the units are of hydrophilic/hydrophobic character. Some researchers demonstrated a method for creating centimeter-scale cell-laden hydrogels through the assembly of shape-controlled PEG micro-gels randomly placed on the surface of a high density hydrophobic solution. The self-assembly process was guided by the surface-tension forces at the liquid–air interface, Oliveira and Mano, 2011.

A simple and inexpensive photolithographic method for surface patterning deformable, solvated substrates is demonstrated using photoactive PEG-diacrylate hydrogels as model substrates. Photolithographic masks were prepared by printing the desired patterns onto transparencies using a laser bio-printer. Precursor solutions containing monoacryloyl-PEG-peptide and photo-initiator were layered onto hydrogel surfaces. The acrylated moieties in the precursor solution were then conjugated in monolayers to specific hydrogel regions by exposure to UV light through the transparency mask. Multiple peptides can be immobilized to a single hydrogel surface in distinct patterns by sequential application of this technique. That opened up its potential use in co-cultures. The human dermal fibroblast adhesion on hydrogel surfaces was investigated to evaluate the feasibility of using these patterned surfaces for guiding cell behavior. Thus, the material properties and photoactivity of hydrogels can be exploited to tailor in desired bio-activity via light-based patterning, Mariah *et al.,* 2006. So the cell adhesion should occur only in the exposed/un-patterned regions of the hydrogel base, resulting in isolated wells of adherent cells. The ability to generate cell clusters separated by topographical barriers is useful in applications where cell outgrowth from the patterned region is unfavorable or where cell guidance via barriers is desired.

THE APPLICATION OF 3D BIO-PRINTING IN ARTIFICIAL SKIN TISSUE

Michael *et al* utilized laser bio-printing technique to create a fully cellular skin substitute. These skin constructs were tested subsequently *in vivo* on the dorsal skin fold chamber in nude mice. The transplants were placed into

full-thickness skin wounds and were fully connected to the surrounding tissue when transplanted after 11 days. The printed keratinocytes formed a multi-layered epidermis with beginning differentiation and stratum corneum. Proliferation of the keratinocytes was mainly detected in the supra-basal layers. *In vitro* the keratinocytes of controls were cultivated at the air-liquid-interface. They also exhibited proliferative cells but rather located in the whole epidermis. Cadherin as a hint for the adherent junctions and therefore tissue formation could be found in the epidermis both *in vivo* and *in vitro*. In both conditions, the printed fibroblasts stayed partly on top of underlying stabilizing matrix from the wound bed and the wound edges in direction of the printed cells. Some blood vessels of the mice wounds could be found growing. In conclusion, the successful cell construct via laser bio-printing and the subsequent tissue formation were showed *in vivo*. These findings represent the prerequisite for the creation of the complex skin tissue consisting of different cells in an intricate 3D pattern, Michael *et al.,* 2013.This was the early important researching results in the field of artificial skin via 3D bio-printing. So it was regarded as the landmark event in the regeneration tissue.

The fibroblasts and keratinocytes were used to be embedded in collagen with 3D bio-printing for skin tissue. Some different characteristics, such as cell localization and proliferation were investigated to study cell functions and tissue formation process in 3D. The researchers further analyzed the formation of adhering and gap junctions, which are fundamental for tissue morphogenesis and cohesion. Specific junctions can be found as cell–cell and cell–matrix connections in all kinds of tissue, abundantly in epithelium like the epidermis. Inter-cellular adherens junctions are fundamental for tissue morphogenesis and cohesion. They are composed mainly of cadherins. Gap junctions allow inter-cellular communication by chemical signals passing through these cell–cell channels. Gap junctions that consisting of connexins are known to play a fundamental role in differentiation, cell cycle progression and cell survival. Thus, cadherin and localization followed by gap junction coupling are good parameters to study tissue properties. It has been shown that cells are not harmed by the printing procedure. Therefore the laser bio-printing is an outstanding tool for generation of multi-cellular 3D skin tissue and its functions, Koch *et al.,* 2012.

The layer-by-layer 3D bio-printing technique was especially suitable for the developing *in vitro* skin of its multi-stratified structure. Lee *et al* presented a method to create multi-layered engineered tissue composites consisting of human skin fibroblasts and keratinocytes which mimic skin layers. 3D free-form fabrication technique on direct cell dispensing was implemented using a robotic platform that prints collagen hydrogel precursor, fibroblasts and keratinocytes. A printed layer of cell-containing collagen was cross linked by coating the layer with nebulized aqueous sodium bicarbonate. The process could be repeated in layer-by-layer fashion on a planar tissue culture dish, resulting in two distinct cell layers of inner fibroblasts and outer keratinocytes. In order to demonstrate the ability of printing and culturing multi-layered cell-hydrogel composites on a non-planar surface for potential applications on skin wound repair, the technique was tested on a polydimethylsiloxane (PDMS) mold with 3D surface contours as a target substrate. Highly viable proliferation of each cell layer was observed on both planar and non-planar surfaces.

The results suggested that skin tissue culture was feasible using on-demand cell printing technique with future potential application in creating skin grafts tailored for wound shape. The printed skin can be directly built on an irregular, non-planar wound surface on-demand fashion, Wonhye *et al.,* 2009. In further study, Histology and immuno fluorescence characterization of 3D bio-printing skin are morphological and biologically representative of human skin tissue. In comparison with traditional methods for skin engineering, 3D bio-printing offers several advantages in terms of shape and form retention, flexibility, regenerative, and high culture throughput, Lee *et al.,* 2014.

Recently the researchers have used 3 D bio-printing technique to print a human bilayered skin using bioinks including human plasma as well as primary human fibroblasts and keratinocytes that were obtained from skin biopsies. They have analyzed the structure and function of the printed skin by histological and immunohistochemical methods, both in 3D *in vitro* cultures and after long-term transplantation to nude mice. In both cases, the generated skin was very similar to the human skin. These results demonstrated that 3D bio-printing is a suitable technology to generate bioengineered skin for therapeutical, Cubo *et al.,* 2016.

FUTURE OUTLOOK AND FURTHER RESEARCHING DIRECTION

3D bio-printing as a novel technique give us some new research ideas in the future. The following discussion would maybe inspire new methods of the artificial skin tissue. During skin would healing, the differentiation of fibroblasts toward myo-fibroblasts and the resulting contraction is a major factor in scar formation. It is also a clinical challenge when skin grafts are employed to replace lost skin due to burns or severe injury. It is interesting to investigate whether and how printed skin grafts can potentially reduce tissue contraction during wound healing by examining the status of fibroblasts differentiation and the micro-architecture of the printed

collagen matrix. The special attention should be given to nanofabrication and electrospining techniques, because they can provide features approaching the nano scale and complexity of the environment *in vivo*, Berthiaume *et al.,* 2011. The electrospining technique supplies a simple and cost-effective method to construct porous scaffolds with uniform fibres in nano scale, Bhardwaj and Kundu, 2010. It may be possible through 3D bio-printing and electrospining to accurately direct stem cell biological behavior. This complex proposal involves the incorporation of cell-matrix, cell-cell, mechanical cues and soluble factors delivered in a spatially and temporally pertinent manner. We can control 3D individual cell micro-environments by incorporating nanotopographic features, Du and Liu, 2014.

The combination of bio-printing and stem cell technology offered the possibility of printing small, developing organoids that would grow into fully functioning organs, Collins, 2014. The application of stem cells within micro-environments may improved transplanted cell viability greatly. Several candidate stem cells include embryonic stem cell, adult stem cell, and induced pluripotent stem cells and so on. The induced pluripotent stem cells can be into adipose stem cells, mesenchymal stem cell, hair follicle stem cell, bone-marrow derived stem cell. Especially the hair follicle stem cell populations provide a further option for artificial skin due to their natural position and roles within the skin. This requires accurate modeling of 3D stem cell interactions within niche to identify key signaling molecules and mechanisms, Hodgkinson and Bayat, 2011. The patient himself can be used as a bioreactor, allowing the small structure to develop into a mature organ. So they may represent an important future resource in the development of dermal substitutes.

The other new techniques include fiber deposition, freeze-drying, gas foaming, salt leaching, precise and piezoelectric technology that can be used for manufacturing 3D scaffolds and cell bio-printing, Li *et al.,* 2016. The unique characteristic of 3D bio-printing skin lies in its form-free shape. It can be made for any shape assisted by computer aided design according to the wound morphology. Moreover, quick turn-around time lends 3D bio-printing to custom fabrication of structures for individual applications. Maybe the artificial skin could match to the shape of a wound geometry or forming tissue-specific replacements grafts post-biopsy or post-surgery, Chang *et al.,* 2011. 3D bio-printing can allow the direct cell deposition in tissue architecture. That technique maybe fabricate a structure that has an accurate anatomical shape, Seol*et al.,* 2014. From the surgical point of view, printed skin tissues have an exciting prospect. Some new techniques like electrospine, nano-materials, stem cell technique will bring further progress in 3D bio-printing. So more efforts should be made on the development of artificial skin tissues.

ACKNOWLEDGEMENT

Special thanks to Professor Kenneth K.Y. Wong in Queen Mary Hospital, the University of Hong Kong. Thanks a lot for his help.

REFERENCES

Bertrand Guillotin and Fabien Guillemot. Cell patterning technologies for organotypic tissue fabrication. Trends in Biotechnology, 2011, 29 (4): 183-190.

Bhardwaj N and Kundu SC. Electrospinning: a fascinating fiber fabrication technique. Biotechnol, 2010, 28 (3):325–347.

Chang CC, Boland ED and Williams SK. Direct-write bio-printing three-dimensional biohybrid systems for future regenerative therapies. J Biomed Mater Res B Appl Biomater. 2011, 98(1):160-170.

Collins SF. Bioprinting is changing regenerative medicine forever.Nat Biotechnol. 2014, 32 (8):773-785.

Cui Xiaofeng, Thomas Boland and Darryl D. D'Lima. Thermal Inkjet Printing in Tissue Engineering and Regenerative Medicine. Recent Pat Drug Deliv Formul. 2012, 6(2): 149–155.

Cubo N, Garcia M and Del Cañizo JF. 3D bioprinting of functional human skin: production and in vivo analysis. Biofabrication. 2016 9(1):015006.

Du Juan and Liu Xuelai. The promising research of 3D bio-printing in skin wound healing. Chinese Journal of Trauma. 2014, 30(10): 1063-1066.

Esmaiel Jabbari. Bioconjugation of hydrogels for tissue engineering. Curr Opin Biotechnol. 2011, 22(5): 655–660.

F. Rengier, A. Mehndiratta, H. Von Tengg-Kobligk. 3D printing based on imaging data: review of medical applications. Int J CARS, 2010, 5(4):335–341.

Francois Berthiaume, Timothy J. Maguire and Martin L. Yarmush. Tissue Engineering and Regenerative Medicine: History, Progress, and Challenges. Annu Rev Chem Biomol Eng 2011. 2:403–430.

Futrega K, Palmer JS, Kinney M. The microwell-mesh:a novel device and protocol for the high throughput manufacturing of cartilage microtissues. Biomaterials.2015, 62: 1-12

Georgios A. Dalkas, Dimitrios Vlachakis, Dimosthenis Tsagkrasoulis. State-of-the-art technology in modern computer-aided drug design. Briefings in Bioinformatics. 2012, 14(6): 745-752.

L. Koch, M. Gruene, C. Unger. Laser Assisted Cell Printing. Curr Pharm Biotechnol, 2013, 14(1): 91-97.

Lee CH, Lee FY, Tarafder S. Harnessing endogenous stem/progenitor cells for tendon regeneration .J clin Invest. 2015, 125: 2690-2701

Lee V, Singh G, Trasatti JP. Design and Fabrication of Human Skin by Three-Dimensional Bioprinting. Tissue Eng Part C Methods, 2014, 20(6):473-484.

Lee Wonhye, Jason Cushing Debasitis and Lee Vivian Kim. Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional free form fabrication. Biomaterials, 2009, 3 0(8): 1587–1595.

Li J, Chen M and Fan X. Recent advances in bioprinting

techniques: approaches, applications and future prospects. J Transl Med. 2016, 20 (14): 271.

Lothar Koch, Andrea Deiwick, Sabrina Schlie. Skin Tissue Generation by Laser Cell Printing. Biotechnol Bioeng, 2012, 109 (7) 1855-1863.

Mariah S. Hahn, Lakeshia J. Taite, James J. Moon. Photolithographic patterning of polyethylene glycol hydrogels. Biomaterials, 2006, 27(12): 2519–2524.

Mariana B. Oliveira and Joa˜o F. Mano. Polymer-Based Microparticles in Tissue Engineering and Regenerative Medicine. Biotechnol Prog, 2011, 27（4: 897–912.

Michael S, Sorg H and Peck CT. Tissue engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skin fold chamber in mice. PLoS One. 2013, 8 (3): e57741

.

Natalja E. Fedorovich, Jacqueline Alblas and Wim E. Henninkl. Organ printing: the future of bone regeneration? Trends in Biotechnology, 2011, 29(12): 601-606.

Seol YJ, Kang HW and Lee SJ. Bioprinting technology and its applications. Eur J Cardiothorac Surg. 2014, 46(3):342-348.

Shamaz BH, Anitha A, Cijayamohan M. Relevance of fiber integrated gelatin-nanohydroxyapatite composite scaffold for bone tissue regeneration. Nanotechnology.2015, 26: 405101

Thankam FG, Muthu J. Alginate-polyester comacromer based hydrogels as physiochemically and biologically favorable entities for cardiac tissue engineering. J Colloid interface Sci, 2015, 457: 52-61

T. Hodgkinson and A. Bayat. Dermal substitute-assisted healing: enhancing stem cell therapy with novel biomaterial design. Arch Dermatol Res, 2011, 303(5):301–315.

Zhang Q, Johnson JA, Dunne LW. Decellularized skin/adipose tissue flap matrix for engineering vascularized composite soft tissue falps. Acta Biomater. 2016:35166-184