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Design and fabrication of human skin by three-dimensional bioprinting

Abstract

Skin has an essential role in preserving homeostasis and in maintaining the safety of the human body from outside environment by playing its role as the largest body part of human. The stratified, categorized and complex arrangement of skin gives a physical protection to the body by maintaining and regulate the transportation of metabolites and water off the body. The injuries that can originate after any chemical or physical trauma can cause impairment of skin barrier and its physiological functions.[1] In skin injuries, considerable amount of skin can be lost, and it develops extremely critical to replace injury caused impaired skin. Transplants in order to protect the lots of water from body and to save the body from opportunistic pathogens can replace the impaired skin. Skin grafts can also expedite the wound recovery procedure and support and restore the barrier and can maintain the regulatory functions on the site of wound.[2,3] Apart from grafts tissue engineered skin plays an exceptionally beneficial role and in vitro stage for the evaluation of skin permeability and adverse inflammation response. The tissue-engineered skin has several advantages in comparison to animal skin by having the major significance by mimicking the skin physiology and easing the ethical concerns of animal use. Additionally, tissue engineered skin models also give the significant insights into the causes of skin diseases, hence, explicate the pathophysiological mechanisms in order to see the progression, and can help in the treatment of skin disease.[4,5] It has been seen that numerous tools have become accessible for the tissue engineering and are adopting different novel approaches and technologies, and amongst these 3D bioprinting offers many significant advantages Since it is possible of dispensing live cells, phase changing hydrogels, insoluble factors and maintaining high cell viability in a desired pattern.[6]

Keywords: skin, bioprinting, tissue engineering

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Rabia Sannam Khan,[1] Qudoos Yousuf[2]
[1]Education and Research Director, ICE Postgraduate Dental Institute and Hospital, 24 Furness Quay, UK
[2]Head of School of Engineering and Creative Sciences, Regent College London, UK

Correspondence: Rabia Sannam Khan, Education and Research Director, ICE Postgraduate Dental Institute and Hospital, 24 Furness Quay, Salford, UK,
Email r.s.khan@salford.ac.uk

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Introduction

Bioprinting uses biomaterials cell factors or cells as bio Ink in order to fabricate tissue structures. The parameters like biocompatibility, cellular microenvironment and cell viability affects the printed product strongly.[7,8] There had been several printing technologies have been explored and improvement has been made in printing different types of tissues liver, cartilage, bone, heart and skin.

Skin

The skin is known to be the largest and major organ of the body and it directly meets the environment. The skin plays a substantial role in the homeostasis and protection from aggressive environment, regulates the body temperatures, balances the water, signal perception, cytokine, hormone, neuropeptide activation and production. It is made of three main layers epidermis, dermis and hypodermis, along with its projections, sebaceous glands, blood vessels, lymph vessels and sensory neurons. The overall skin has several cells and extracellular matrix. Extracellular matrix, growth factors and skin cells are the rudimentary components for skin regeneration.[5,9]

Skin is therefore; more prone to the damages caused by environment and specifically burn injuries. Most commonly, burn injuries accounts for more than one billion per year worldwide.[10] Burned skin is followed by the damage caused by heat, radiation, electricity, or chemicals and severe complex conditions can occur in deep and widespread burns.[11,12] These complications can cause bacterial infection that can lead to sepsis, hypovolemia that can cause shock and leave to contraction of scarred tissue due to inappropriate healing of wound. The damage to the skin causes the death of the cells of the skin, dehydration due to the loss of the body fluids, electrolyte imbalance, and circulatory and renal failure. The most dangerous vulnerability of skin burn wounds is the infection because the skin becomes more predisposed to bacteria's and pathogens because of losing the integral layers of the skin. Hence, it is critical to cover the burn injuries and in order to replace the proteins lost, fluids administration intravenously to balance dehydration and ultimately to save patients life in severe cases. The survival rate of burn patients has been seen to improve greatly in the past decade due to the application of grafts.[12–15]

Clinical need for skin regeneration

The regeneration process of skin is a systematic process and includes classic phases of hemostasis, inflammation, proliferation, and angiogenesis and maturation. Approximately more than 11 million people get affected by burn related injuries the burn injuries occurrence is seen mostly in domestic low to middle-income countries, armed conflicts, industrial accidents also contributes in the higher incidence of burn injuries.[16,17] Although due to the increased prevention serious burn injuries had ben decreased the mortality rate attention has been shifted towards recovery of burn injuries. The worldwide demand of tissue regeneration and organ replacement is at its increase steadily.

3D printing

3D printing has transformed tissue engineering because it allows the creation of complex constructs by layer-by-layer fabrication. Bioprinting an emerging computer assisted technology, is capable

of assembling tissues layer by layer by accurately positioning the biological materials, living cells, along with the 3-D control of functional components. The aim of bioprinting is to develop an engineered organ or tissue in an organized, mechanized and optimized manner. Groll et al redefined the strategies of bioprinting and suggested that, "a construct is bioprinted when living single cells, bioactive molecules, biomaterials or cell-aggregates small enough to be printed are used for fabrication".[18] There had been several techniques and biomaterials were being utilized in order to print biological constructs in various different sizes, shapes and resolutions. Bioprinting application can be used in several research areas such as stem cell, skin, cartilage, bone, heart valve, or in cancer research etc. The most common bioprinting techniques includes the selection of material sources of cells with the purpose of design strategies towards 3D bioprinting.[19–21]

Advancements in regenerative medicine and tissue engineering has created the possibilities to regenerate the damage organs and tissues back to the functional organ or tissue by taking the support form 3D bioprinting. Bioink is the main component for 3D printing and the bioinks properties are vital when considering their selection since they are critical in the development of functional organs and tissues.[22–25]

Bioprinting

The loss or failure of organs causes the limited supply of them globally hence; it becomes a critical and costly issue in health care system. Therefore, tissue-engineering research specifically in the construct of cell scaffold microenvironment so to promote the restoration of different types of tissues including cartilage bone cardiac tissue tendon and skin is essential and is the need of an hour. Scaffolds are known to be the vital component for tissue restoration because they give the physical structure for the cells to transplant, mechanical support, and for the maintenance of physiological functions.[6,26] A suitable and satisfactory scaffold must have certain mechanical properties, geometric structures, cell populations, chemical gradients and properties where they can assist the growth of various types of tissues and cells. When it comes to ideal bone scaffold in tissue engineering, it has the most favourable cytocompatibility, it gives surface did the sales so that they can adhere, can proliferate, can differentiate, and can secrete extracellular matrix.[27]

3D bioprinting of tissues and organs

3D bioprinting was firstly developed in 1986 by Charles W. Hull and was known as "stereo lithography". 3D printing has the ability to manufacture large-scale cost-effective tissue engineered printing. A variant of 3D printing is 3D bioprinting which deposits the living cells altogether with 'bioink' (hydrogel-based scaffolds) by allowing the designing of separate individual components of organ or tissue and in this manner facilitates the complex tissue formation. The biological constructs are fabricated through 3D printing by the layer-by-layer material addition onto the scaffold so as to build up the 3D tissue with having the CAD file input. Therefore, bioprinting allows to alter the CAD file suitably before printing. Commonly, five steps had been involved in the 3D bioprinting, firstly, scanning/imaging of the target tissue, secondly, the development of model by using the image, thirdly, the selection of scaffold and cells depending upon the selected tissue to be printed, fourthly use of bioprinter to print the tissue and lastly, allowing the bio printed tissue to get mature. Bioprinting can be performed in vitro or in vivo and generally, bioprinting process include three major stages, which includes tissue pre bioprinting, bioprinting and maturation, or post bioprinting stage.[28–31]

Skin bioprinting

A unique methodology used to reconstruct the skin had been studied and investigated by several studies. Some of the major advantage of using bioprinting for skin fabrication in comparison of using the conventional engineering strategies such as cell culture on scaffold and bioreactor maturation, which includes long process and production time bioprinting can achieve similar results with improved aspects. The two approaches for skin bioprinting used are in vivo and in vitro, whereas, in vivo bioprinting has many advantages for burn reconstruction that includes precise cells deposition on wound, decreases the need for multiple surgeries, long processing time, and less expensive from in vitro bioprinting. However, in vitro bioprinting is permitted to mature in bioreactor after it gets transferred onto the wound site. Bioprinting enables re-epithelization and wound closure effectively.[6,19,32,33] Cubo et al. reconstructed the skin with keratinocytes and fibroblasts printed from human plasma.[34] In another study, laser assisted bioprinting was used in order to print keratinocytes and fibroblasts in order to construct simple skin.[35]

Skin bioprinting stages and bioinks used for skin bioprinting

Skin bioprinting involves three key phases of skin pre-printing, bioprinting and skin maturation. The first stage of skin pre-printing includes to isolate the cells, expansion, differentiation of cells and bioink preparation (made of biomaterial or cells) from skin biopsy. In healthy skin the process can be followed as it is but for the injured skin stem cells might be needed for the differentiation and can be achieved from adipose, perinatal, mesenchymal and induced pluripotent stem cell sources.[36]

The second stage of bioprinting, the precise information print 3D geometric files are converted into Stereolithography (STL) file having the synchronizes for print head path. These files have the information for the use of reconstructing the 3D model and are constructed by CAD-CAM or can be created from the images taken clinically from Magnetic Resonance Imaging (MRI) and computed tomography (CT) imaging. By dividing the STL model in to the layers and bioprinter tool path creation, pathways for the print heads are established. Each slice from its thickness the resolution of printer can be determined (usually it is in the range of 100–500μm). Furthermore, the bioprinter deposits the bioink by reading the STL file layer by layer in order to build up the 3D organ or tissue from the series of 2D slices. To have high fidelity, high quality image is required for bioprinting and clinical images and image processing tools can be used to extract the in vivo information, cell distribution and skin geometry.[37–40]

The last phase of skin bioprinting, which is the maturation stage, is highly significant and crucial in the event of in vitro bioprinting. It instantly follows the printing and skin constructs are delicate. As well as they need a bioreactor to be matured in before they can be used for transplantation.[41]

Photocurable gelatin and its bioprinting applications

Gelatin is one of the complex gels, which has experimental difficulties due to its rheological properties. Gelatin has printing complexities when it comes to 3D printing. Gelatin is a biomaterial also known as macrophage activator and fibroblast attractant, has the ability in the promotion of epithelialization and granulation tissue formation. The strategies used are temperature dependent. In addition,

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blending gelatin with other polymers results in extra costs and might not be calibrated with every device. Tayebi et al. presents different methods of printing Gelatin and examines rheological properties of gelatin. The author has employed a 3D printing technique using gelatin as ink as a innovative design for potential usage of tissues engineering. The author has also described a design criterion to create a membrane that could potentially be used for oral mucosa.[42,43] Gelatin based hydrogels, have unique features with biocompatibilities, non-immunogenicties and rapid biodegradability's in skin 3D bioprinting technologies for instance gelatin/hyaluronan, gelatin/fibrinogen and gelatin/alginate/fibrinogen.[44] Skin bioprinting has enormous capability in the construction of tissue with best outcomes in burn patients. The early the intervention, the reduction of potential infections with faster healing reduction in scarring and better outcomes cosmetically can be expected. Further advancements in the 3D printer's biocompatible bioinks will facilitate the great use of this technology. On the whole, 3D bioprinting a transformative expert technology for the use of wound reconstruction and will ultimately lead to the paradigm shift in the outcome of patient's health.

The bioprinter that can be used usually in laboratories are shown in the Table 1 below; we have in our laboratory would have used one nozzle 3D bioprinter for the gelatine preparation.

Table 1 3D printing of organs by using gelatine based hydrogels[44]

No	Technology for 3D bioprinting	Bioink design	Uses
1	**One nozzle extrusion-based 3D bioprinting**	Gelatin/hepatocyte	hepatic tissues
		Gelatin/hepatocyte/ chitosan	hepatic tissues
		Gelatin/hepatocyte/ alginate	Hepatic and cartilage tissues
		Gelatin/hyluronan	Repair of brain defect
		Gelatin/alginate/ adipose derived stem cell (ADSC)	Vascular networks
2	**Two-nozzle extrusion-based 3D bioprinting**	Gelatin/alginate/Fibrinogen/ADSC/ alginate/fibrinogen/hepatocyte	Vascularized liver an adipose tissues
		Gelatin/alginate/fibrinogen/ ADSC-gelatin/ alginate/fibrinogen/ pancreatic islet	Cardiac tissues and vascularized hepatic and adipose tissues
		Gelatin/alginate/fibrinogen/ HepG2; gelatin/alginate/fibrinogen/ hepatocyte or gelatin/alginate/ fibrinogen/hepatocyte/ADSC	Anti-cancer drug screening and in vitro liver tumor model
3	**One-nozzle extrusion-based 3D printing**	Gelatin/alginate/myobalst	Muscles
4	**one-syringe extrusion-based 3D printing**	Gelatin/alginate/smooth muscle cell / aortic valve leaflet interstitial cell	Aortic valve conduits
5	**Multiple cartridge extrusion-based 3D printer**	Polycaprolactone gelatin /fibrinogen/ hyaluronic acid/glycerol	Bone Skeletal muscle and cartilage
6	**Envision TEC 3D Bioplatter**	Polyethylene glycol/gelatin-PEG/ fibrinogen	Grid strictures for cell seeding

The review represents the in-depth literature review of the preliminary studies on the fabrication of human skin by the use of 3D bioprinting technology. Photocurable Gelatin was chosen as they have unique features with biocompatibilities, non-immunogenicties and rapid biodegradability's in skin 3D bioprinting technologies for instance gelatin/hyaluronan, gelatin/fibrinogen and gelatin/alginate/fibrinogen. The optimisation of this platform can expedite the construction of human skin intricate model consisting of adnexal and secondary structures. The complex models will allow the most essential advancements of understanding of skin in order to engineer the better wound grafts, transdermal and topical development of tools which will ultimately reduce the animal reliance. The approach and protocols described here can be used for the translation into designing the disease models such as psoriasis, allergic contact dermatitis, atopic dermatitis, vitiligo and malignant skin models including melanoma in order to discover and develop the significant therapeutics against such skin diseases. Future work will involve for additional advancement and manufacturing of individual skin that integrates various skin cells, and secondary structures as well as adnexal.

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None.

Conflicts of interest

None.

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