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Skin Bioprinting: An Innovative Technology Forskin Reconstruction

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ABSTRACT
3D bioprinting is an advanced technology that can easily create skin grafts for the patient by using different biomaterials and cells in less time and cost. 3D bioprinting can fabricate skin tissue which can help to reconstruct skin in severe skin disease and burn patients. This technology improves the production process of the covering skin that covers the entire burn bound. With the help of this technology, scientists can create skin transplants of specific shape and size as per patient's requirement and which is also suitable for the patient's injury.
Keyward: 3D bioprinting, skin grafting, reconstruction of skin

I. INTRODUCTION

The largest and complicated organ of human body is skin. Skin is the outermostcovering of human body which protects the muscles, ligaments and variousinternal organs. Skin acts as the first line of defense hence, any injury firstattacks the outermost layer of the body. So, various regeneration methods ofskin tissue is necessary. Day by day need for organ donors are increasing.
Using3Dbioprintingregenerationoforgansandtissues arepossibleinthelaboratory (Ai L & L. Weng,2013). Skin wounds are common which may result from trauma, skin diseases, burn or removal of skin during surgery (Coyer et al., 2015). Nowadays, skin injuries due to burns is a verycommoncase.Even minor defects bring psychological distress on the affected individuals. There are several options of skin tissue engineering such as autografts, allografts, xenografts etc. Though each technique has some demerits like creation of secondary wounds, risk of immune reactions etc. Everyyear,around11millionpeopleneedmedicalhelp. Theburninjuries are life-long ache whose survival rates are high among the patients (M. Bacakova&Musilkova J, Riedel T et al., 2016). In this regard, tissue engineering holds great promises for improving the treatment of skin defectsand it is a viable method in the tissue and organ reconstruction (J. B. Jank et al., 2017). Tissue-engineered skin (TES) is mainly composed of biomaterials, cells, and bioactive factors. It can completely cover skin wounds, accelerating wound healing and promoting the vascularization of dermal substitutes. However, there are also many limitations such as non-pigmented skin, insufficient elasticity of dermis, long-term postoperative scars, and loss of skin appendages etc. (T. Weng et al., 2021). Therefore, these limitations of TES have been resolved by the development of three-dimensional (3D) printing technology with its accuracy and high resolution.

Bioprinting is a process where biomaterials are used to create tissue likestructures which looks similar to the original tissue. 3D bioprinting is a type of Additive Manufacturing (AM) technology that becomes widely used in the medical sector for reconstruction of the burn injuries with the help of computer-aided design (CAD) model inputs.This technology helps to create better skin grafts which is also cost effective (P. He et al. & P. Rider et al., 2018). Inkjet 3D printing and laser assist 3D printing is used commonly. In future, this technology can create 3D tissue-engineered structures that can rectify the defect in a patient-specific organ (Md. Javaid & A. Haleem, 2021). Though it involves somerisks such as developing cancer, teratoma etc. Bioink is used to create thesestructureslayerbylayer (L. Bai, D. D. Ginty& A. Zimmerman, 2014).Inbioprinting,mostlyuseoflivingcellsareencouragedwhereasin3Dbioprintingmostlyplasticisusedtomakethemodels. Three dimensional bioprinting works on the principle of deposition ofbiomaterials

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layer by layer in the infected area (H. Bien, C.Y. Chung & X. Zong,2005). 3D bioprinting is used to develop complicatedorgansandtissueswhichlooksverysimilartotheoriginalorgansandtissues (A. Chaudhari et al.,2017).
The3basicstepsof3Dbioprintinginvolves(C.M. Chuong et al.,2012):

- Pre-printinginvolvesimagingofthetargettissue,
- developmentwithCAD/CAMsoftwaresandselectinga biomaterial
- Post-printinginvolvesmaturationand implantationoftissues

Within 5-7 years,the bioprinting market will increase by 15.7% and by 2025 it will cross $4.70billion. With the advent of skin bioprinting it will mark the end of the testing ofdrugs on animals (3). It is a promising technique with the aim to produce 3Dtissues or organs. Skin bioprinting involves the replacement of skin injury with skinsubstitutesbytheprocessofreconstruction.

II. SKIN BIOPRINTING – AN UNORTHODOX APPROACH

Overtheformerdecennium,therehasbeenan outstandingadvancement towards the evolution of substitutes which are in vitro-engineered. These invitro-engineered substitutes help to imitate human skin. It either acts as an aidto grafts for the substituting lost skin, or for the initiating in vitro model.Anewanduniqueplanofactionhasevolvedknownastissueengineering.Thishasprogressedbyutilizingthecontemporaryadvancesin diverse areas. These fields of action include stem cell research, bioengineering,polymer engineering and nano medicine. Lately, a growth in the area of 3Dprinting technology and its advancements are being used for a larger benefit.This is popularly known as bio printing. This helps to formulate cell loadedscaffoldswhichinturnfabricatesmaterialsmore complimentarytotheoriginal,indigenous tissue. Bio printing works on smoothening out the process of theconcurrent and highly unequivocal skin cells deposition of multiple types andbiomaterials.Thisisaprocedurewhichrequiresadvancementtowardstraditional tissue-engineering of skin. Bio printed skin replaces or acts as acounterpart to equivalents consisting of dermal as well as epidermal elements.Suchconstituentsputforwardahopefulperspectiveinthefieldofskinbioengineering. Numerous mediums which include either naturalor syntheticbiopolymers and cells, in addition to or without adding a warning towardsmoleculessuchasgrowthelementswhichareb eingavailedtoassembleeffective skin constructs. This applied science makes an impressive appearanceas a fresh and unique policy plan to prevail over the topical constrictions in theengineeringofskintissuesuchasestablishmentofs weatglands,weakvascularization, andnon-appearanceofhairfollicles.

Advantages

- Growth components, extracellular matrix as well as units which are epidermal can be easily located in the necessary places which makes them extremely reproductive.
- Affability, extensibility, inflated yield and improved plasticity.
- We can mark and reprint the matrix of blood vessels to make it much more remarkable for an extended endurance of the operation.

Disadvantages

- The cost is very much high. It needs expensive biological printers and manpower.
- Bio printing technology has not yet developed enough. Bio printed skin constructs may originate some security complications, more so, if it is applied straight away to clinical implementation.

III. WOUND HEALING AND BIOPRINTING

Skin is a complicated and most sensitive part of our body hence, if there is any wound or burn injury we need to treat it immediately. Skin bioprinting treatment by darning of wounds diminishes gap. But it is difficult to treat a patient with extensive burns. With age skin tends to become thin and sensitive. Hence, wound healing becomes a tough job. Skin biotechnology is one of the promising technique which involves the use of it in various medicinal lines such as growth of tissues and cells in laboratory etc. Skin bioprinting helps in the treatment of wounds by darning wounds and alleviating, lessen the chance of contamination, reduce blemishes, upgrade cosmetic consequences etc.

There are different kinds of bioprinting technologies. Among them four of which are widely used at present: Inkjet-based printing, Extrusion-based printing, Laser-assisted printing and DLP-based printing—dynamic optical projection stereolithography (DOPsL).

There are two basic styles for skin bioprinting - In vitro and in situ bioprinting.L.

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Koch et al., (2012) reported that 20 layers of fibroblasts (murine NIH-3 T3) and 20 layers of keratinocytes (human immortalizedHaCaT) embedded in collagen were printed by a Laser-assisted BioPrinter to generate simple 3D skin which is similar with dermis and epidermis (Fig. 2). In 2013, V. Lee et al. demonstrated that the 3D Printed skin samples on collagen layers retained their form and shape, whereas manually deposited structures shrank and became concave shapes. Separately Michael et al. (2013) demonstrated that bi-layered constructs formed dermis and epidermis. After 11 days of transplantation, some blood vessels from the wound bed could be observed.

In case of in situ bioprinting, amniotic fluid-derived stem cells (AFSCs) and bone marrow-derived mesenchymal stem cells (MSCs) were suspended in fibrin-collagen gel, mixed with the thrombin solution and then printed onto the wound site. The bioprinter was used to deposit two layers of a fibrin-collagen gel by depositing a layer of thrombin, a layer of fibrinogen/collagen, a second layer of thrombin, a second layer of fibrinogen/collagen, and a final layer of thrombin.

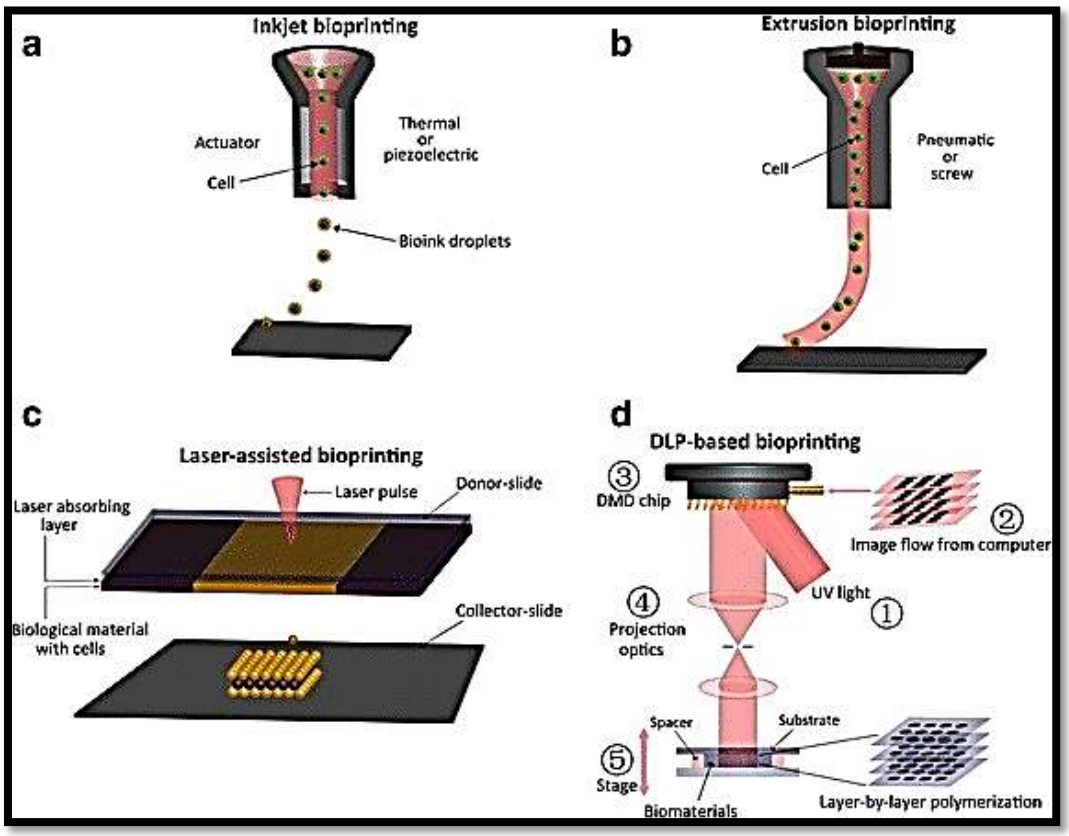

Fig1: Bioprinting techniques. **a.** Inkjet bioprinter eject small droplets of cells and hydrogel sequentially to build up tissues. **b.** Extrusion bioprinter use pneumatics or manual force to continuously extrude a liquid cell–hydrogel solution. **c.** Sketch of the laser printer setup. **d.** Schematic of the DLP based bioprinter—dynamic optical projection stereolithography (DOPsL).

(**Picture curtsy:** P. He, J. Zhao, J. Zhang, B. Li, Z. Gou, M. Gou, X. Li Bioprinting of skin constructs for wound healing.Burns Trauma, 6 (2018), p. 5)

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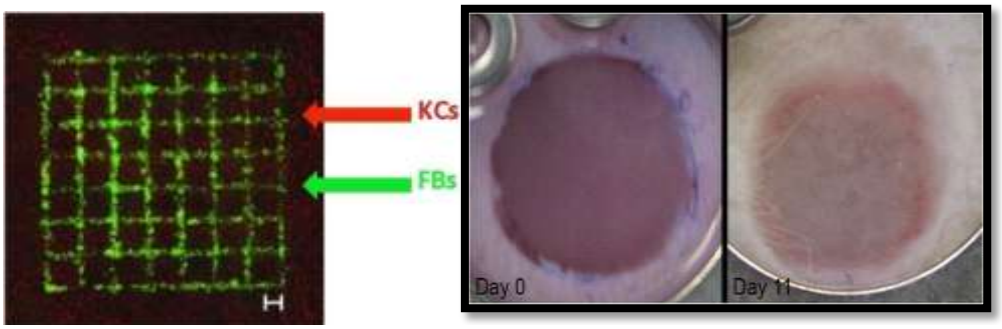

(a) (b)

Fig2: In vitro bioprinting.

a.Fibroblasts (green) and keratinocytes (red) was printed by the laser printing technique.
(**Picture curtsy:** Koch L, Deiwick A, Schlie S, Michael S, Gruene M, Coger V, et al. Skin tissue generation by laser cell printing. BiotechnolBioeng. 2012; 109(7):1855–1863. doi: 10.1002/bit.24455.).
b.Skin construct inserted into the wound directly after the implantation (day 0) and on day 11.
(**Picture curtsy:** Michael S, Sorg H, Peck CT, Koch L, Deiwick A, Chichkov B, et al. 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.)

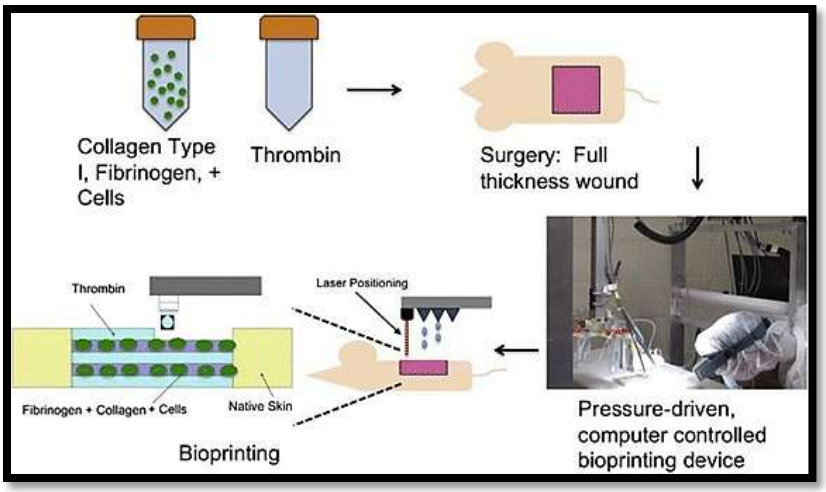

Fig3: In situ bioprinting.

(**Picture curtsy:** Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl Med. 2012; 1(11):792.)

IV. BIOINK & CREATING BIOINK

The bioinks are the most important ingredient for 3D bioprinting. It is used forthe development and regeneration of various organs and tissues. An ideal bioink should have few physicochemical properties, such as proper mechanical, rheological, chemical, and biological characteristics. Itisamixtureofcells, biomaterials,growthfactorsandnutrients.In3Dbiopri ntingmainlytwotypesofbioinkbiomaterialsareused:

Natural biomaterials used as bioink

Hydrogels-based bioinks are biocompatible and typically biodegradable. Hydrogel biomaterials include alginate, gelatin, collagen, fibrin/fibrinogen, gellan gum, hyaluronic acid (HA), agarose, chitosan, silk, decellularized extracellular matrix (dECM), poly(ethylene glycol) (PEG), and Pluronic. As collagen is the main structural protein in the extracellular matrix (ECM) of mammalian cells, several scientists used collagen as bioink.Gelatin is one of the most widely used natural polymers for its thermo sensitivity and ability to form a hydrogel at lower

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temperatures. For bioprinting applications, gelatin has been used as a bioink and/or as a composite with other polymers.Gelatin-alginate composite bioinks also used for bioprinting (T. Zhang et al., 2013). Alginate was also widely used as a bioink in the LaBP method. In tissue engineering, fibrinogen and fibrin are mainly used to construct functional tissue for the replacement of damaged tissues for wound healing. They are biocompatible, biodegradable, non-immunogenicand they also induce cell attachment, proliferation, and ECM formation (T. Rajangam, 2013, X. Cue & T. Boland, 2009). Silk is a naturaI Polymer and it has long been utilized as a scaffoIding material for both soft and hard tissue engineering applications.Gellan gum is an anionic polysaccharide produced by bacteria. Like alginate, it forms a hydrogel at low temperatures when blended with monovalent or divalent cations (AH Bacelar et al., 2016). Dextran and agarose are natural polysaccharide that has been widely used in tissue engineering applications

Synthetic biomaterials used as bioink

PEG is a synthetic polymer synthesized by polymerization of ethylene oxide which faciIitate the bioprinting processes. PEG with reactive groups (PEGX) is a valuable tool to modify the bioink's properties and to increase the bioink options. Pluronic is a type of poloxamer which has good printability and temperature-responsive gelation. Due to these properties, it is well-suited for use in bioinks (C. C. Chang et al., 2011).

There are a few commerciaI biomaterials that have been recentIy introduced such as Dermamatrix, NovoGeI, CELLINK etc. Recently nanomaterials [e.g. silver nanoparticles (AgNPs), gold nanorods (AuNRs)]have been used for producing conductive bioinks (P. S. G. Ozkerim et al., 2018).

V. CHALLENGES

An updated applied science becoming more and more evident for assemblingartificialskin is the 3D bio printing mechanization. Nevertheless, there still existsomeremarkabletechnologicaldisputesintheoccurrenceofbio-mimeticpracticalskin forclinicalimplementation.

A single question which is majorly looked out on skin bio printing is the one ofbio ink. The fundamental units of original, local skin are the quantity-seedingunits. In spite of the recent up gradation in cell culture methodology for givingrise to cells for bio printing, concerns are still present as to whether there areadequate units which can very well be created willingly for bio printing of skinestablishmentforclinicalutilization.Thepotentialityofcellsresentduringtoday's recent times, could be sustained in biological substances, but suchmediums have the need of bio-elasticity of the raw and original skin. Thesemediums which are acceptable, not only for 3D scaffold impression but also forseeding units. These also include the electrophysiology of the nude skin whichacts in a much better way for skin bio printing. Hence, developments of all thefacts and figures used to engrave scaffolds have become a crucial provocationforfutureanalysis.

VI. APPLICATION

Bio printing necessitates the application of 3D printing mechanism to formepithelial tissues as well as organs. This operation has been tested and tried ondiverse research spheres incorporating transplantation, grafting and clinics, useofprogenitorcellstoproducetissuessynonymously appliedtothetermregenerative medicine, research on carcinoma, drug testing along with drugscreening and (HTS) high-throughput screening enabling the testing of largenumbersof chemicalsubstancesforactivityindiverseareas ofbiology.

VII. FUTURE

Inthenearfuture,theappliedsciencebasedon 3Dbioprintingcouldextendanaspiration amongst people. At present, these people count on donor organs.Falseorgansprintedbyavailingbioinkcreatedfromcellsbelongingtoapatienthimselfcouldabolishtherequirementofatransplantaltogether.Thiscouldalsodoawaywiththerequirementfororgandonorsandbringingdowntheprobablethreatoftransplantrejection.Crucialevolutionintheoperationoftissueswhich are 3D bio printed could be expected for the following 10–15 years. To beginwith, this works by concentrating on simple, uninvolved prototype tissues fordrug screening and cosmetic testing. This operation is backed by an expandingnumber of experiments on animals and clinical investigation of 3D bio printedmuscle or epithelial tissue in the coming 10 years.We can expect a great dealofexcitingpossibilitiesintheupcomingtransplantationprocedures.Someofthemore updated possibilities comprises of the transfer of a vascularized humanbody part containing multiple tissue types (such as skin, muscle, bone, nervesand blood vessels), conventions authorized the fortunate deprecation or evencessation of amantadine or immunosuppressant drugs, and the application ofbody's raw materials for organ restoration. Availing the facilities of bio printingwill qualify the integration of different

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varieties of cells in the membrane whichincludes foramen (sweat) and oleaginous or sebaceous glands as well as hairfollicles. This in turn will sanction the renewal of Keratinocytes or in a simplerlanguage,ourskintissue,withtheformationan dcellularconstructionbearingaresemblancetothenati veorindigenous tissue.

VIII. CONCLUSION

Thetechnologywhichhasmadeanimmensei mpactbecauseofitshugecapability to make a smoother fabrication of anatomical and body relevanttissue as well as qualifying improved, more compatible practical solutions incases of burn patients is none other than the skin bio printing technology. Theapplication of bio printing in cases of skin restoration after burns is definitelypromising. Bio printing authorizes an error-free placement of the innumerableoriginalskincellclassificationswithallmi nutedetails.Italsoenablesmanufacturing clonable constructs to restore bruised or damaged skin. Theapplicationof3Dbioprintingforrelievingmutilati onmakessuchhealingsmuchfaster, which is analytical in cases of large-scale, serious damage caused due toburning.Anadvancedandinitialmediationwilllesse nthepossibilitiesforsepticemia and provide lesser scarring, secured and speedy healing, as well asmuch superior cosmetic after-effects. This will also come up with a decrease inthequantityofsurgeriesessentiallyneededandthelon gdurationforwhichthepatients require to stay behind in the hospital. To make clinical translationsuccessfully possible by taking advantage of the benefit of bio printing forwound restoration, the damaged product evolved needs to be uncomplicated.The injury should also be able to integrate in an uninterrupted manner into thesurgicalprocedureandtheoperativeactivity.Morea dditionalprogressinterm of occurrence of clinical grade 3D bio printers in a systematized approach andbiocompatible or microporous bio inks will allow broader utilization of thistechnology in surgery. Universally, these all-inclusive facts help us to comprehend that 3D bio printingis an extremely life-changing technology, and its application for reformation ofwoundwillactasarevolutionaryaswellasfundament alchangeinconsequencesofallpatients.

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