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3D Bio-Printing: An Emerging Technology for Skin Regeneration

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Abstract

Skin is the largest organ of the body and injury to this organ is frequently exceptionally serious which should be dealt with. Skin transplantation is the most customary strategy utilized on account of harmed skin however it has a few restrictions. 3D bioprinting is a novel system for the age of human tissues. It includes the layer-by-layer affidavit of biomaterials on the harmed region and recovery of lost tissue. In skin bioprinting, utilitarian skin substitutes are created. In bioprinting, bio-inks and biomaterials are utilized to make tissue frameworks. Distinctive bioprinting procedures are utilized in the printing of human tissues. A few methodologies are utilized in skin bioprinting for example *in situ* and *ex vivo*. Both these methodologies vary just in the site of development. Specialists have made a few examinations on skin bioprinting. In this review, we will talk about various bioprinting procedures, the choice of cells in bioprinting and various wellsprings of bio-inks.

Keywords: 3D bioprinting; Bio-ink; Reconstruction; Scaffold; Skin transplantation

Introduction

Skin is a significant organ of the body. All out body weight includes 15%-20% of the skin. It performs numerous significant capacities for example secures against microorganisms, heat, UV beams and looks after homeostasis. It goes about as a hindrance to the outer condition. Skin and its limbs by and large make the integumentary framework [1-3]. Skin is the body's first line of resistance [4]. Skin is a perplexing organ. It comprises of three layers; hypodermis that is greasy, dermis that contains fibroblasts enrooted in extracellular grid with vessels and hair follicles and epidermis made up of keratinocytes [2]. At the point when skin is harmed because of consumes, injury or other skin wounds, it is important to unite skin [5]. It is evaluated that 265,000 passings are brought about by consumes comprehensively [6]. Skin is a tissue-designed by utilization of allografts however it has a few impediments [7]. The complex and non-uniform nature of skin blocks to make a total structure of the skin. Some assembling conventions that structure a skin model with full-thickness are accessible [8]. An ordinary tissue-designed skin build is comprised of keratinocytes embedded on a 3D collagen Fibroblast (FB). FBs are blended in with hydrogel and are crosslinked to make the FB-

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dermal layer. The develop is then refined and adult skin is gotten after air-fluid interface culture [8,9]. In like manner tissue designing methods, tissue models have numerous difficulties regarding fixed measurements, significant expense, and less applications. 3D bioprinting is a rising field in tissue designing that has gotten fundamental in creating fake tissue models by saving numerous layers of cells and biomaterials. 3D cell printing is alluded to as a manufacture stage in which biomimetic tissue models are framed [10]. 3D printing has been utilized to frame numerous biomimetic structures. It is significant in both tissue building and regenerative medication. For making skin builds utilizing 3D printing procedures, a few examinations have indicated that collagen with fibroblasts and keratinocytes can be imprinted in an attractive way [8]. Bioengineered skin can be produced using both normal and engineered polymers. 3D approaches have been embraced in skin tissue designing since they can create complex structures [5]. The biomaterial utilized in 3D cell printing is bio-ink containing cells that are fused into 3D builds with alluring shape. Numerous materials have been utilized as bio-ink. In an examination, it was indicated that the Decellularized Extracellular lattice (dECM) gives cells condition that is like the earth of living cells [11]. In another investigation, another bio-ink dependent on GelMA-collagen was detailed by doping with tyrosinase. It is appeared by *in vivo* tests that injury recuperating rates were quickened when this bio-ink was utilized [12]. 3D bioprinting has gotten progressed as of late and has gotten one of the most rising innovation in tissue substitution. This innovation centers around the age of composed structures that are like local tissues. Numerous structures extending from not many to several micrometers in size can be produced in hydrogels utilizing 3D bioprinting. Along these lines, it is conceivable to make numerous layers of the skin including dermis, epidermis, hypodermis, sweat organs and hair follicles. At the point when tissue designing innovation is joined with 3D systems, it lessens a few issues like tissue dismissal, poor mending and pathogen move [7].

In this survey, we examine and abridge the ongoing methodologies in 3D bioprinting. A short presentation is given about 3D printing innovation in skin transplantation. We talk about the biomaterials, bio-inks and other manufacture techniques. Diverse cell-printing strategies have been talked about. We talk about regular and engineered polymers utilized as bio-inks. This survey gives a short review of 3D bioprinting of skin (**FIG. 1**).

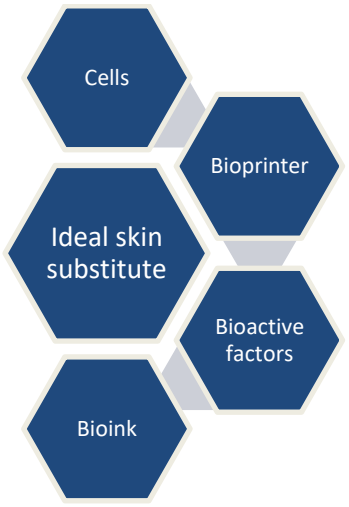

FIG. 1. **Requirements of 3D Bioprinting for skin regeneration.**

3D Bioprinting

3D bioprinting is a procedure wherein there is layer by layer affidavit of biomaterials to create a 3D structure. This strategy was first evolved in 1986 by Charles W Hull. It is initially called stereolithography [13]. Bioprinting is the creation of 3D builds

includes 5 stages: target tissue imaging, improvement of a model utilizing, CAD-CAM programming; cells and biomaterial platform are picked relying upon the sort of tissue, bioprinter is utilized and tissue is printed, development of bio-printed tissue. There are 3 phases of the procedure of bioprinting: pre-bioprinting, bioprinting, and post-bioprinting [13] (**FIG. 2**). In pre-bioprinting, cells are secluded from a skin biopsy, extended, separated and bio-ink is readied which is comprised of cells and biomaterials. When printing cells for sound skin, essential cells are utilized while when there is a harmed skin, foundational microorganisms are utilized. Bioprinting includes the transformation of print records containing precise data of 3D geometry to the Stereo-Lithography document (STL). The unpredictable 3D structures can be remade utilizing CAD-CAM programming. STL documents are perused by bioprinter and 3D tissues are built from 2D structures by ceaselessly keeping bio-ink. Post-bioprinting is otherwise called the development stage. It is the last phase of bioprinting. On account of *in vitro* bioprinting, skin builds are developed in bioreactors while *in situ* bioprinting includes development at the site of injury [13]. Three principle approaches are utilized in 3D bioprinting these days: expulsion based bioprinting, laser-based bioprinting, and bead based bioprinting. The technique is chosen dependent on the properties of cells and biomaterials utilized and qualities of cell/tissue that will be printed [3,7]. In expulsion based bioprinting, the solenoid-based framework is utilized to launch the bio-ink arrangement. It permits a more extensive determination of biomaterials however it is less precise and gives lower goals than different systems. In any case, in this procedure, high consistency materials can be printed [14]. In laser-based bioprinting, a laser is utilized. It depends on laser-prompted forward exchange. This is a spout free method. It gives higher goals and has lower throughput. In any case, the fundamental hindrance is that it is more slow than different strategies. In bead based bioprinting, spouts are utilized and bio-ink drops are stored. It has fast however it needs less-thick bio-ink [3,7].

Effect of immunomodulation, stromal vascular fraction treatments, micro RNA (miRNA) and small interfering RNA (siRNA) based skin therapies have also been demonstrated in 3D printing [15].

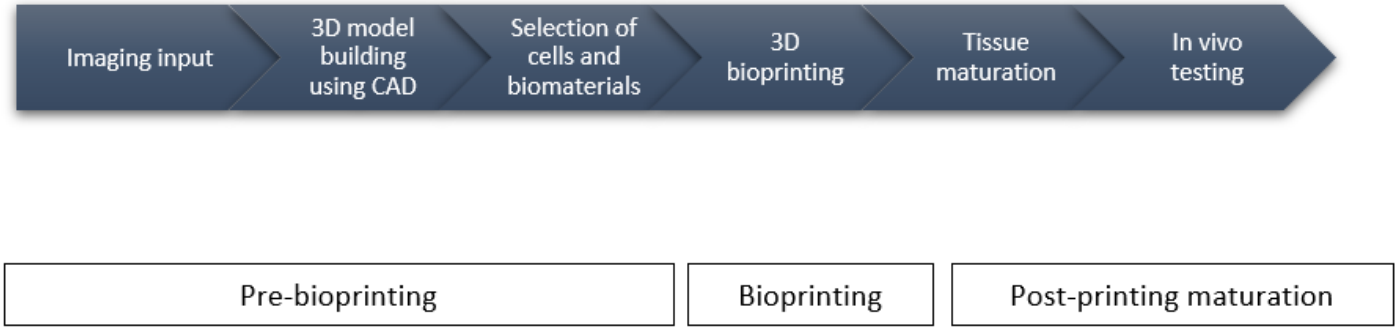

FIG. 2. **Process of bioprinting (steps).**

Ex Vivo and *In Situ* Techniques of Skin Bioprinting

Till now, it has been researched that skin bioprinting is a novel technique to remake useful skin tissue. 3D bioprinting can be utilized for enormous scope creation of skin tissue develops. These builds can be utilized in toxicological and pharmaceutical testing [16]. This is on the grounds that it is a normalized and computerized strategy to develop useful skin builds requiring little to no effort.

There are two primary procedures in skin bioprinting: *ex vivo* and *in situ*. These methodologies are distinctive in the site of printing and development of tissue [15,17]. In the *ex vivo* strategy, tissue develop is bio-imprinted *in vitro* and afterward developed and

separated. After development, it is transplanted at the site of injury. Ng et al. [9] manufactured 3D pigmented skin develops *in vitro* by utilizing a two-advance bioprinting technique. These builds were acquired by utilizing 3 skin cells, keratinocytes, fibroblasts and melanocytes. These 3D bio-printed builds were contrasted with pigmented develops acquired by manual-throwing. It has been accounted for that 3D printed builds indicated more like credulous tissues than physically framed tissues [9]. *In vitro* models rely upon multistep manufacture strategies. Notwithstanding, Byoung et al. built up another method to create a 3D build in a solitary advance utilizing a trans-well framework [10]. *In situ* bioprinting includes cell bioprinting straightforwardly on location of injury. The cells are pre-refined and permit skin development in the harmed zone. Seol et al. [5] built up an *in vivo* model to reenact skin tissue on the human face. Skin tissues were produced on complex facial injuries was appeared by histological assessment through *in vivo* examination utilizing 3D BioMask [5]. In an examination, Ding and Chang [6] proposed *in situ* bioprinting procedure to unite skin onto an apparition consume wound bed. They introduced a condition of-workmanship treatment for wound mending. Picture guided *in situ* bioprinting has empowered to create persistent explicit skin unions to treat wounds in less time [6]. Full-thickness Skin Fold platform (SFS) was created with a perfusable vascular pedicle by perfusion decellularization. This fold platform was biocompatible, had acceptable mechanical properties and indicated regenerative potential *in vitro* and *in vivo* [18] (**FIG. 3**). Scaffold-free bioprinted strategy was also used to construct 3D skin. In this stud, adult ear was constructed in 3D environment resulting in rapid differentiation of dermis. This approach was useful but it takes long time in maturation [19].

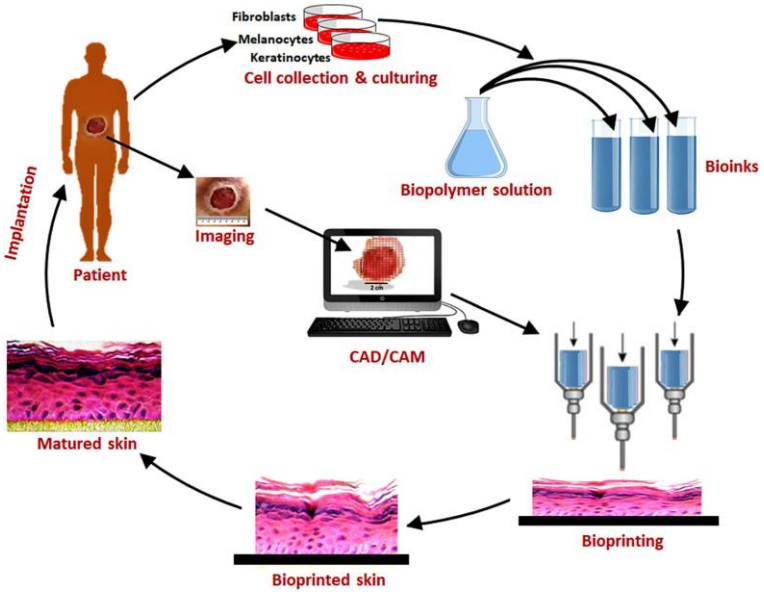

FIG. 3. **Steps involved in fabrication of 3D bioprinted skin.**

Selection of Cells in Skin Bioprinting

The determination of suitable cells is a main consideration in bioprinting for skin regenration. Keratinocytes (KCs) and Fibroblasts (FBs) are the most widely recognized cell types used to create skin tissue builds. They form epidermis and dermis individually. In an experiment, human Bone-Marrow-Derived Mesenchymal Stem Cells (hBM-MSC) and human Nasal Chondrocytes (hNC) were utilized to produce a full-thickness skin graft. It was seen that skin graft survived when exposed to external environment [20]. In another reearch, it has been reported that full-thickness biomimetic skin can be printed by utilizing keratinocytes, fibroblasts, and melanocytes on air-liquid interface culture [21]. Fibroblasts and human dermal microvascular endothelial cells were used to engraft bio-printed tissue [22]. A facial mask was developed by utilizing keratinocytes and fibroblasts [5]. Multilayered skin grafts have also been produced utilizing keratinocytes, fibroblasts, Human Endothelial Colony Forming Cells (HECFC) and human placental

Pericytes (PCs). It was shown that 3D bioprinting can be utilized to build vascularized human skin *in vitro* that is similar to naive skin [22]. Neonatal human dermal fibroblasts and neonatal ordinary human epithelial keratinocytes were also cultured and afterward bio-printed for tissue development [2]. Different structures, for example, skin members, melanocytes, endothelial cells, and sebaceous organs could be co-printed to form fully functional skin. Biomimetic skin was produced for wound dressing using oil-infused Polydimethylsiloxane (iPDMS) and silver nanoparticles (Ag-Np). As silver has anti-bacterial properties so it was best approach for anti-infection and wound healing [23].

Bio-Ink used in Skin Bioprinting

Bio-ink is a basic segment in 3D skin bioprinting. Bio-ink ought to have the accompanying properties: biocompatibility, biodegradability, solid, bioinert and pliable. There are 2 kinds of bio-ink: characteristic biopolymers and manufactured biopolymers. Characteristic biopolymers are normally created as proteins and polysaccharides. Proteins incorporate gelatin, collagen, fibrinogen, egg whites, and thrombin, and so on and polysaccharides incorporate chitosan, cellulose, and chitin, and so forth. Manufactured biopolymers are created misleadingly that are Polyglycolic corrosive (PGA), Polylactic corrosive (PLA), Poly Lactic-Co-Glycolic corrosive (PLGA), and so on. Be that as it may, numerous bio-inks and biomaterials are utilized however biocompatibility is significant for choice. Common biopolymers are for the most part favored over engineered biopolymers. Different natural prerequisites ought to be satisfied by bio-ink during and in the wake of bioprinting [13,14]. Seol et al. figured composite hydrogel-based bio-ink for 3D bioprinting [5]. Nanocomposite hydrogels/platforms were likewise utilized structure 3D structure [24]. In an investigation, bio-ink was framed utilizing a decellularized Extracellular lattice (dECM). The dermis was treated with catalysts and skin dECM was removed. The 3D develop was made by stacking bio-ink made out of dECM with human fibroblasts in the spout of bioprinter [11]. The significant wellspring of the bio-ink plan is collagen type I since it is exceptionally biocompatible. As of late, a blend of gelatin, alginate, and fibrinogen was utilized to figure a cross breed bio-ink. dECM has been broadly utilized as the most reasonable wellspring of bio-ink in 3D printing innovation. Byoung et al. exhibited the potential S-dECM bio-ink through *in vitro* and *in vivo* applications [10]. Apelgren et al. made bio-ink utilizing Bacterial Nano-Cellulose (BNC) by utilizing ACC (Aqueous Counter Collision) strategy. The ACC method made BNC exceptionally appropriate biomaterial for 3D printing and it was accounted for that cell-loaded 3D-bio-printed ACC-BNC is a great answer for fix ligament [25]. Shi et al. created another bio-ink containing Gelatin methacrylamide (GelMA) and collagen with Tyrosinase. Tyrosinase-doped bio-ink was framed by a two-advance crosslinking technique. It was inferred that Ty-doped bio-ink can build stable 3D structures and would be helpful for wound mending and skin recovery [12].

Conclusion

3D bioprinting is the most encouraging innovation that includes the manufacture of useful tissue builds by printing cells and biomaterials at the same time. The main considerations influencing the bioprinting procedure are the determination of cells and biomaterials and the utilization of reasonable bio-ink. Skin bioprinting includes the utilization of cells and biomaterials to develop useful multilayered skin through *in situ* and *ex vivo* approaches. Numerous kinds of biomaterials are utilized for example characteristic and engineered polymers. Numerous kinds of bio-inks are utilized with various sorts of cells for example keratinocytes, fibroblasts, melanocytes, and undifferentiated organisms. The utilization of skin bioprinting in wound mending and to supplant harmed skin would be an incredible activity in skin substitution treatment. This method is significant promotion in facial plastic medical procedure. It will decrease the danger of diseases and will build wound recuperating rates. It will likewise bring about fewer skin medical procedures and will lessen the hour of treatment. Specialists have produced 3D skin develops that

are utilitarian. Be that as it may, there are likewise a few restrictions for example development of skin limbs, veins, and so forth. Researchers are attempting to manufacture veins and other skin limbs alongside the dermis and epidermis.

To put it plainly, 3D bioprinting is an extraordinary innovation in the remaking of skin with an enormous number of points of interest. Later on, it will open more open doors in the transplantation of harmed, injured or consumed skin.

Conflict of Interest

There is no conflict of interest in this paper.

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