Advances in the Development of Tissue Engineering Applied to the Skin Using Three-Dimensional Bioprinters for the Treatment of Burn Patients

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ABSTRACT

The concept of three-dimensional printing was developed in 1980, and the idea of three-dimensional printing to manufacture objects was established in 1986. In 1993, the technology allowed the printing of plastics and metals, with a series of three-dimensional printers having different applications being subsequently developed. In 2002, Italian researchers developed the dermal regeneration matrix (hyalomatrix) with hyaluronic acid and silastic fibers, mimicking the epidermis. In 2003, Mexican researchers developed an allogeneic keratinocyte culture as a system for the release of growth factors in skin lesions. Over the past 10 years, 15 matrices of dermal regeneration have been developed. This technology has had a great impact on engineering and medicine. In medicine, an important application is tissue engineering, not only for the manufacturing of skin and grafting but also for conducting scientific investigations on the evaluation and discovery of drugs. The use of bioprinting of tissues can allow a layer-by-layer aggregation of cells to be obtained, allowing organization of multiple cell types in a desired structure. After in vitro cell culture, allowing respective growth and maturation to achieve the desired tissue, tissue implantation can then be performed. The three-dimensional bioprinting technique can improve both spatial resolution and reproducibility, allowing optimal conditions for cell incubation and maturation. In addition, with a limited supply of donors, bio-fabrication of organs and tissues can help with future transplant procedures.

KEY WORDS: *Cell reproduction, Innovation, Skin*

INTRODUCTION

The skin is important for maintenance of homeostasis and for providing protection from the external environment.[1] It provides a barrier to the entry of xenobiotics into the body. In addition, the skin regulates the transport of water and metabolites outside the body. The use of three-dimensional (3D) engineering applied to the skin has advanced along with the development of biological techniques and in vitro models. Three-dimensional engineering allows clinicians to obtain a stratified matrix where several tissues can be manufactured according to the respective function and different sizes.[1] The most basic 3D model to represent the skin uses the epidermis and dermis layers.

CELL CULTURE, METHODS, AND MATERIALS

Keratinocytes and fibroblasts can be used as constituent cells to represent the epidermis and dermis, respectively. Collagen can be used to represent the dermal matrix of the skin (the scaffold structure).[1] Some printing techniques can print the scaffold (or dermal matrix) together with the cellular material; other techniques print the scaffold first and then add the cellular material. The process is to first print layers of collagen and then a layer of fibroblasts and/or keratinocytes; this process can be repeated several times.[1]

As mentioned, the most basic structure to represent the skin is 2 layers: the epidermis and the dermis. The epidermis is represented by keratinocytes (for the stratum corneum, corneocytes are used). The dermis is represented by synthetic substrates (nylon and polycarbonates) or protein stratifications (such as collagen, glucosamine, and fibrin) or dermis from dead skin or fibroblasts. Fibroblasts

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are the most used to represent the dermis among these substrates.

Incubation consists of growing (culturing) keratinocytes (or corneocytes) in an air-liquid interface in a dermal substitute. The process of incubation and exposure of the epidermis to an air-liquid interface can achieve maturation and stratification of the skin (or formation of the stratum corneum). One technique to culture cells includes growing fibroblasts and keratinocytes (with addition of trypsin and keeping on ice before printing) at a temperature of between 35 °C and 40 °C, with a solution of CO_2, penicillin, and bovine serum.[1] During culture, the medium is changed every day for approximately 2 weeks.[1]

Keratinocytes receive nutrients from the lower surface of the culture during a culture period of 3 to 4 weeks, although the culture medium may need to be occasionally changed during this period.[1] Cell viability and cell interactions can be analyzed through a microscope.[1] In this way, it is possible to obtain a tissue that mimics the biological, physiological, and morphological characteristics of the human skin. This process can also reproduce the skin structure. However, improvements are needed with regard to cellular interactions (coupling) and immune functions (functionality) of the skin.

Factors added to improve tissue maturation during culture include human keratinocyte supplements, hydrocortisone, bovine insulin, ascorbic acid, and calcium chloride. Currently, the hydrogel is under investigation to represent the dermal matrix. The dermal matrix is the base impression material, where keratinocytes and fibroblasts are added. Hydrogel (diluted with a phosphate-saline solution and kept on ice before printing) is used as a collagen and as a scaffold material for printing.

Inkjet pressure (range, 1-3 psi) and the duration of the printing pulses (range, 500-1000 μs) (separation between cells of 100-1000 μm) are important for cell viability, resolution, droplet volume (range, 20-60 nL), and cell interaction. These parameters are determined from the viscosity of the biomaterials that are printed. Sodium bicarbonate vapor can be applied at the interface of each collagen layer and between the first collagen layer and the support glass disk. This procedure is done to increase adhesion between the layers.[1]

THREE-DIMENSIONAL BIOPRINTERS USED FOR TISSUE REPRODUCTION

Three-dimensional printers are an advantage over the use of animal skin (due to ethical aspects), can be used to mimic the physiology of the skin, and can be used to study skin disorders. Use of tissue made from 3D printers is also an advantage over conventional tissue grafts with regard to reproducibility and high yield levels. Three-dimensional bioprinting can be considered as a simultaneous deposition of cells (keratinocytes, corneocytes, fibroblasts [epidermis and dermis]) and other additive factors in a dermal matrix (scaffold structure; hydrogel).

To obtain the optimal printing parameters (air pressure, speed, and vertical movement [z offset of the injector]), preliminary studies and analyses are often needed. These studies are used to find appropriate results for cell viability and densities, concentrations (1-5 mg/mL for collagen, 0.5-10 million cells/mL for cell suspension), and percentage of cells in the epidermis and dermis. In addition to air pressure (inkjet), other parameters include pulses of printing duration (for inkjet/drop printing), size of the printing layer, volume of the drop, space between drops/resolution, and cell density.[1] Resolutions for bioprinting techniques range from a scale of a few micrometers to a little beyond 100 μm.[2] The types of printing include lithography, laser, ink-jet deposition, and photopolymerization.[2]

Strategies used in tissue bioprinting include biomimicry, autonomous assembly, and microtexture.[3] Another application is to obtain superficial grafts (as bandages) for wound healing (there is also a spray that helps heal superficial wounds) and to study skin disorders. It is important that the bioprinting method does not produce toxicity or collateral damage to the cells and their DNA, and the bioprinting method must result in a mechanically and functionally stable tissue.

There are 2 techniques: one that involves incubation and one without incubation (performed in the operating room, called clinical bioprinting). For the incubation technique, cell viability in vitro must be shown before tissue integration, with good specific tissue function.[2] Currently, hydrogel as a collagen and printing base material is under study for the development of functional tissues. Although two-dimensional (2D) and 3D environments can be used to produce biological activity for cells, tissues and organs require a 3D scheme.[2]

It is possible to perform a manual (conventional) assembly of 2D cell sheets or layers (layer-by-layer printing) for a 3D structure. Conventional tissue manufacturing involves cell culture within a scaffold that has a porous structure to mimic the properties of the extracellular matrix.[4] This technology using scaffold (or extracellular matrix) has been used to make bones, skins, and cartilage. However, it has complications with regard to mimicking complex tissue structures and for locating multiple types of cells in desired and ordered positions.[4]

For tissue engineering and the manufacturing of functional tissues and organs for respective transplant to be successful, a reasonable time scale is needed.[2] With 3D

printing, efficiency, automation, reproducibility, and scalability (micrometer resolution) can be obtained. The respective functionality of the tissue must be checked over a wide range of external conditions (including pressure, temperature, and exposure to the atmosphere) and its coupling without collateral effects after the tissue is implanted. These conditions must also be verified in vitro. Another technique used to improve tissue functionality is the 2 photon polymerization[2] technique.

The bioprinting technique consists of 3 stages: software development (with the use of computers) for tissue or organ design and the control of cell deposition, printing (cell and hydrogel deposition), and maturation of the tissue or organ (incubation or the use of a bioreactor). This last stage involves the use of biological techniques of cell assembly and fusion or aggregation.[2] After this, the respective implantation of the tissue in the skin can be performed. However, it is also possible to perform tissue implant in the skin without an incubation process (clinical bioprinting).

During software development, images of the anatomical structure of the tissue (via computed tomography or magnetic resonance imaging) are needed; a computer automated design (CAD) program is then used to transfer the image to a bioprinter. The CAD software (eg, AutoCAD, SOLIDWORKS, CATIA) converts the image into a blueprint. The blueprint is converted to a heterogeneous model where the materials to be used and the cellular composition and distribution are described.[4] These programs transform the image into 2D cross sections (layers) of appropriate scale (adequate resolution). The printing device can then add them layer by layer.[3]

The printing stage involves a specific printing method and the use of a combination of materials or other additives.[3] It consists of the simultaneous deposition of cells using a deposition technique in a layer-by-layer mode.[4]

The last stage involves the incubation process or the use of a bioreactor to grow the cells (the maturation process) before the tissue is implanted.[2,4] However, most current bioreactors are not appropriate to recreate the functioning of the tissue after the maturation process. Therefore, it is necessary to refine the bioreactor technology or use another incubation technique.[3] However, as mentioned, tissue implantation can also occur without incubation (clinical bioprinting).

The aggregation of cells (keratinocytes, corneocytes, fibroblasts) in hydrogel is currently under study. It is thought that hydrogel may improve the assembly of tissues and organs.[2]

THREE-DIMENSIONAL PRINTING TECHNIQUES

The different types of 3D technology for printing are inkjet 3D bioprinting, microextrusion 3D bioprinting, and laser 3D bioprinting laser; within the laser technique, there is also stereolithography.[3] These types of printers are not in competition, but their application depends on the scale required. That is, if the need is for basic research of cell analysis and without tissue construction, laser printing is indicated.[5]

Inkjet three-dimensional bioprinting

Early studies have used a modified 2D inkjet printer to print layered tissues. This is a noncontact technique that uses thermal forces or piezoelectric, pneumatic, or electromagnetic microvalves to print bioink drops to a substrate. This method allows users to replicate a design made on the computer.[3] Of inkjet printers, the thermal design is the most used. In addition, thermal inkjet printers are also easier to maintain than piezoelectric printers.

With inkjet printers, cells and biomaterials are modeled toward certain substrates (structures) in the form of drops,[4] and a structure is formed by means of the continuous deposit of many drops in specified locations by using the computer software. The duration of local heating in each printing drop is for a short period of time (2 μs), temperatures are increased at 4 °C to 10 °C, and a local temperature at 300 °C is easily obtained.[3]

One of the advantages of this technique is the low cost and high spatial resolution. However, disadvantages include drop size (resolution) compared with other techniques.[3] This technique can also show impressions of low viscosity and cell distortion. The viscosity can be minimized using biomaterials based on water.[5]

Thus, the main characteristics are electromagnetic, thermal, or piezoelectric forces that emit successive drops of bioink toward a substrate as well as its high speed, availability, low cost, loss of precision in the location of drops and size, necessity for low viscosity, and cell viability of around 85%.[3]

Microextrusion three-dimensional bioprinting

The microextrusion 3D bioprinter uses mechanical or pneumatic forces to print the bioink material. Printing occurs by contact through small, continuous flow of bioink material, achieved through CAD software and computer. At present, microextrusion 3D bioprinting is the most used and the most common method.[3] The printing is of high viscosity such as complex polymers, and printing can result in a high cell density. However, a disadvantage is cell distortion (due to tension among cells), low resolution, and loss of cell viability.[3]

Thus, the main characteristics are mechanical or pneumatic forces that dispense bioink through an injector, high viscosity of bioink, high cell density, distortion of cell structure, 40% cell viability, and low cost.[3]

Laser three-dimensional bioprinting

The laser 3D bioprinter is a noncontact technique that sends laser pulses through a tape containing bioink, with cells suspended at the bottom of the tape. The tape is then vaporized by the laser pulse, resulting in discrete drops that adhere to the substrate. This step is repeated several times until the 3D structure is created.[3]

One of the main advantages of this technique is the high degree of precision and resolution (1 cell per drop). This technique gives rise to the possibility of printing DNA and microcellular arrangements[3] and also allows printing with a very high cell density and high resolution. For example, a bioink concentration of 10^8 cells/mL can be used to print discrete drops containing at least 1 cell. Disadvantages include low cell viability compared with other techniques and the preparation time of the tape. However, printing time is fast if the preparation time of the tape is not considered.[3]

Thus, the main characteristics are the bioink and cells suspended at the bottom of a tape, which, when vaporized by a laser pulse, are dispensed to a substrate. Other characteristics include the high degree of precision and resolution, ability to use high viscosity, printing with high cell density, large printing time, 95% cell viability, and high cost.[3]

Laser three-dimensional stereolithography

Laser 3D stereolithography uses photopolymerization, where the laser or ultraviolet light is directed toward a photopolymerizable liquid polymer, which converts the polymer into a layer. Because each layer is polymerizable, it is repeated several times to form the 3D structure.[3] When acrylics and epoxies are used as a photopolymerizable material, they result in high manufacturing accuracy compared with other techniques. However, the disadvantages are the intense ultraviolet radiation that is needed, the slow maturation process after incubation that is required, and the few compatible materials for use with this technique.[3]

Thus, the main characteristics are its use of digital light to dispense the bioink in a layer-per-layer form, adequate accuracy, low printing time, high intensity ultraviolet light, slow processing (after incubation), loss of material compatibility, 90% cell viability, and low cost[3] (see Figure 1).

PRINTING MATERIALS

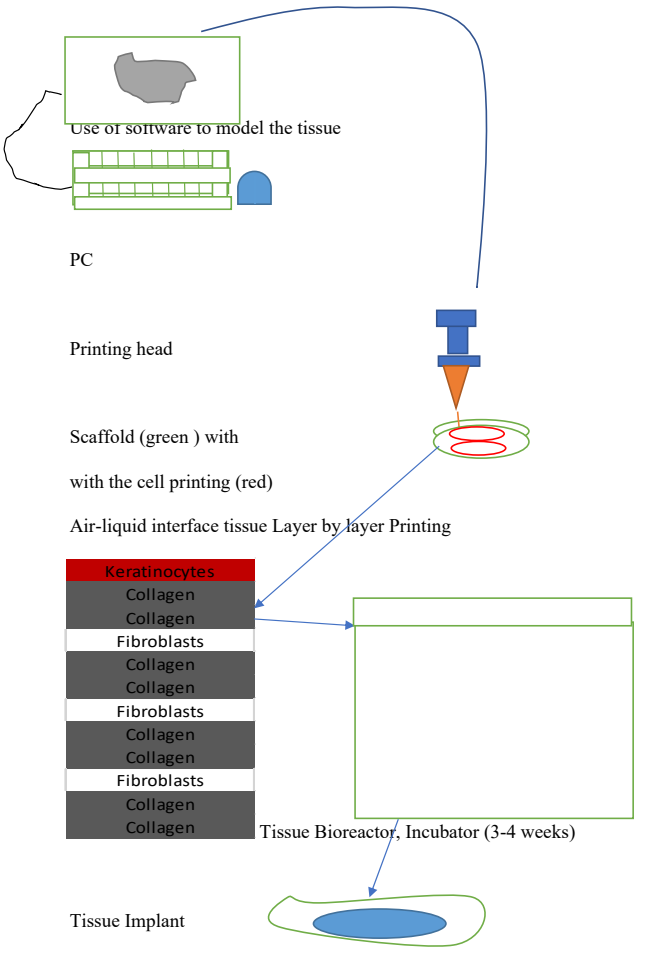
The printing materials should have the following characteristics: appropriate viscosity, high mechanical forces, a structure that should not collapse during the layer-by-layer deposition process, biocompatibility, and biodegradable. The biomaterials can be either synthetic or natural. Synthetic biomaterials have the disadvantage of cell proliferation and differentiation, whereas the natural biomaterials are mechanically weak.[4]

Polyethylene glycol (PEG) is one of the most used synthetic polymers for printing.[4] It is a water-soluble polymer widely used for cell encapsulation. Polyethylene glycol can be modified with acrylic groups such as diacrylic ethylene glycol (PEGDA). A copolymer based on poly-methacrylamide lactate and PEG has also been developed as bioprinting material. The disadvantage of PEG is that it is a nonbiodegradable polymer. Biodegradable synthetic polymers include polylactic acid, polylactic-lactic-glycolic acid, and poly-e-caprolactone.[4]

Among the natural biomaterials, collagen is an important protein of the extracellular matrix.[4] Collagen consists of 3 amino acids: glycine, proline, and hydroxyproline. Cells can adhere and proliferate in collagen. Collagen has been widely used to regenerate skin, bone, and cartilage[4] and is widely used for 3D printing. Disadvantages include the weak mechanical force and long-lasting coupling (cohesion) of the tissue.[4]

Gelatin, a derivative of native collagen, is not stable at body temperature. However, gelatin can be modified

FIGURE 1. Three-Dimensional Printing Schematic



by adding methacrylate group to the gelatin[4] (GelMA); GelMA hydrogels have been printed to create cartilage and cardiovascular structures.[4]

Another natural biomaterial, hyaluronic acid, has a high biocompatibility and biodegradability[4]; hyaluronic acid hydrogels can be formed through modification with the methacrylate group when they are exposed to ultraviolet light. The result has a strong mechanical force ideal for printing hard tissues such as bones.[4]

Fibrin is a natural protein, and fibrin gels have been printed to improve vascularization. Vascular networks and tubular tissues are printed by using fibrin gels.[4] Alginate, a natural polysaccharide (algae), is biodegradable and bioinert and is excellent for printing long tissues.[4]

Despite these advantages, most hydrogels are weak. Thermoplastics can be deposited with these biomaterials during printing to improve the mechanical strength of hydrogels.[4] Examples of thermoplastics include hydroxymetiglicolide-co-e-caprolactone added with a methacrylate group (through photopolymerization), allowing resistance to axial and rotational forces to be improved.[4] Ceramic or glass particles can be incorporated to be added to the scaffold of the matrix. These particles mixed with hydrogels reinforce the mechanical forces, improving bioactivity.[4]

Cells removed from tissues and organs (by using physical or biological methods) (de-cellularized matrix) are excellent materials for printing because they have bioactive signals from native tissues. These cells mimic the composition of tissues.[4]

A summary of materials used for biofabrication of tissues and organs through 3D bioprinting is shown in Table 1.[4]

Bioink

During the bioprinting process, bioink is printed layer by layer. Bioink is made of cellular materials, additives (eg, growth factors, molecules), and a support scaffold (the extracellular matrix).[3] Among the different bioink components used for bioprinting are synthetic scaffolds, natural scaffolds, and hydrogel scaffolds.[3]

The specific properties of bioink depend on the mode of printing, the type of tissue, and the cell concentration for a given bioink. For example, inkjet prints require low viscosity, low thermal conductivity, and low temperature. On the other hand, extrusion prints can withstand high viscosities, but these present the problem of possible cell distortion.[3]

A balance must be achieved with regard to obtaining the required respective structural property without affecting cell

TABLE 1. Summary of Materials Used for Biofabrication of Tissues and Organs Through Three-Dimensional Bioprinting

Tissue or Organ	Biomaterial	Cell Type	Application
Tissue construction	Alginate, gelatin, agarose, calcium salts of polyphosphate	Sarcoma cells of human osteogenic	High rate of cell proliferation, Young modulus reduced
Scaffold of osteochondral tissue	PCL, alginate	Osteoblasts, chondrocytes	Mechanical properties improved, high cell viability
Osteochondral models	Gelatin, methacrylamide, and PLA microcarriers	Chondrocytes, osteoblasts	Better mechanical properties, high cell viability
Atrial cartilage	Collagen	Chondrocytes	Specific morphology of the patient, live neocartilage formation
Human skin	Collagen	Fibroblasts, keratinocytes	Good retention, high cell viability, good morphological and biological imitation of the human tissue
Aortic valve	PEGDA + alginate	Aortic pig VICs	High cell viability, heterogeneous mechanical forces
	Alginate-gelatin	Aortic pig VICs, human aortic roots, SMCs	High cell viability, phenotype retention, good cell dispersion
	Methacrylate HA and GelMA	HAVICs	High cell viability
Bionic ear	Alginate + chondrocytes (ear), AgNP-silicone (antenna-coil)	Chondrocytes	High cell viability, neocartilage formation
Similar tissues	Decellularized cartilage tissues, heart tissues, adipose tissue	hASCs, hTMSCs	High cell viability, ECM formation
Neural cellular structures	Collagen, fibrin gel	Hippocampal and cortical cells, NTw neuronal cells	Healthy cellular electrophysiological structures

Abbreviations: AgNP, silver nanoparticles; ECM, extracellular matrix; GelMA, gelatin plus methacrylate; HA, hyaluronic acid; hASC, human adipose tissue-derived stem cells, HAVICs, aortic valve interstitial cells; hTMSC, human tubal mesenchymal stem cells; NTw, neuron time warp; PCL, poly-e-caprolactone; PEGDA, polyethylene glycol modified with diacrylic; PLA, polylactic acid; SMC, smooth muscle cell; VICs, valve interstitial cells

viability.[3] Therefore, there is an optimal concentration range of bioink, where increased cell concentrations can affect the cell viability, affecting the cell migration and diffusion.[3]

Bioink scaffolds

Scaffolds should provide cells with secure fixation and protection from the mechanical and thermal stresses of printing, as well as support cell growth and proliferation without affecting the cell phenotype.[3] Biocompatibility is the biggest limiting factor for scaffolds. They should be cytologically compatible without causing immune responses, inflammatory responses, or premature differentiation of the stem cell.[3]

Hydrogel scaffolds

Of scaffolds, the hydrogel scaffold is the one most used at present. Hydrogel scaffolds, which can mimic the extracellular environment of tissue and absorb water, are moldable polymers produced from a wide range of components, such as collagen, fibrin, algae, and other materials.[3] For example, they are used in contact lenses and biological adhesives such as polyethylene glycol polymer.[3] Hydrogel scaffolds are also used to form aortic valve ducts. There are 2 techniques regarding their use: they can be printed first and then cells are deposited (keratinocytes, corneocytes, fibroblasts), or they can be printed with the cells already suspended in the hydrogel matrix.[3] For example, a bionic ear could be constructed by means of the hydrogel matrix by placing algae (alginate) with corneocytes around an inductive coil antenna, allowing electromagnetic signals over a wide frequency range to be received.[3]

Hydrogel scaffolds are composed of hydrophilic polymers linked through covalent bonds or through intermolecular attractions. The hydrophilicity allows easy exchange of gases and nutrients and high biocompatibility and allows easy modifications. These scaffolds have low mechanical properties and cell culture (sediment). The viscosity is adjustable by adding other factors.[3]

Synthetic scaffolds

Examples of synthetic scaffolds include those made of PEG, such as PEG diacrylate and polyacrylamide gels.[3] The advantage of these bioinks is the ability to manipulate their physical and chemical properties, but a disadvantage is the low cellular interaction (coupling). Synthetic scaffolds also do not effectively mimic the biological environment of the tissue.[3]

Synthetic scaffolds are derived from both natural and synthetic sources (PEG, with low viscosity, PEGDA, and polyacrylamide gels). The synthetic scaffold is easily modifiable, composed of functional groups, and allows for nonimmunogenicity.

Natural scaffolds

Natural scaffolds include polymers such as gelatin, collagen, fibrin, algae (alginate), and other natural polymers.[3] They have high cell viability, biocompatibility, and cell growth compared with synthetic scaffolds. A disadvantage is that natural bioink scaffolds do not support remodeling and elasticity compared with synthetic scaffolds and have limited ability for modification.[3] For example, gelatin and alginate have low resolution; however, natural and synthetic bioink compounds can be formed to improve the resolution.[3]

DIFFERENTIATION OF STEM CELLS DURING PRINTING

One of the biggest advantages of 3D printing is the ability to influence the differentiation of stem cells during multiple stages of the process.[3] Selection of the stem cell source, the printing method, the choice of scaffold, additive factors, and the incubation process can influence the differentiation of stem cells toward a specific target of the tissue. The use of stem cells can also influence the immunotolerance and expansion once it is implanted in the tissue.[3]

Sources of stem cells

The 3 main sources used are embryonic stem cells (high degree of multipotency but has ethical and immunogenicity aspects), mesenchymal stem cells (can stimulate immunotolerance with low degree of multipotency), and pluripotent stem cells (has high degree of multipotency but promotes tumorigenesis according to some studies).[3]

Printing method for stem cells

Tensions in each printing technique can influence the differentiation of stem cell. For example, mechanical pressure with inkjet printing can influence differentiation of the mesenchymal stem cell to form cartilage and bones. Shear forces in extrusion printing can influence differentiation to endothelial tissues and bones. Laser printing can preserve multipotency.[3]

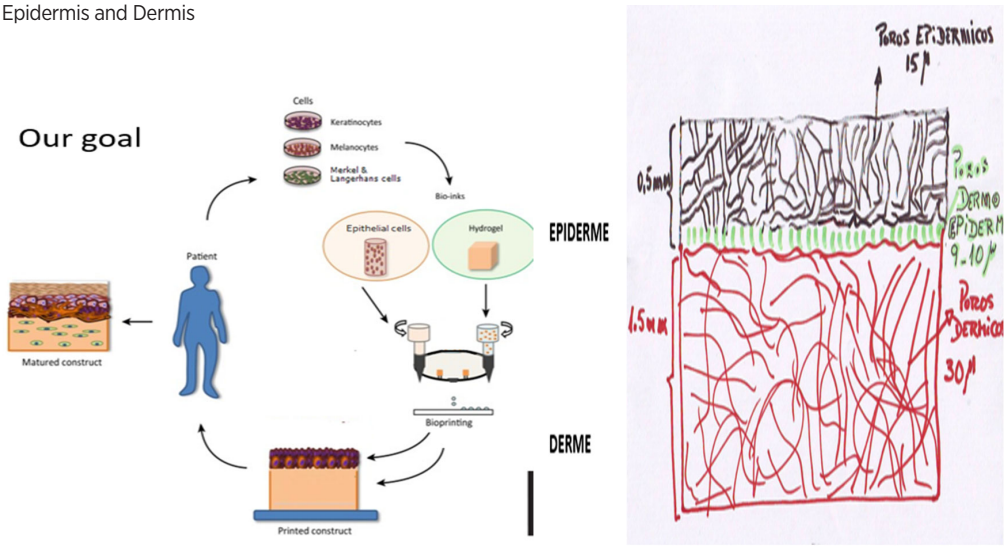
Additive factors for stem cells

Factors must be added to the bioink before printing or to the printed tissue before the maturation process.[3] These factors influence the differentiation of stem cells. Examples of these factors include fibroblast growth factor, platelet-derived growth factor, and morphogenetic proteins; other factors that influence differentiation of stem cells include dexamethasone and ascorbic acid.[3] Small spheres of polymers (microcarriers) have been shown to promote the differentiation when they are added to the bioink, allowing for adhesion and fixation.[3]

BIOPOLYMERS

Hydrogels and biopolymers are not always suitable for printing methods. One option is to combine substances to maximize the usefulness of polymers.

FIGURE 2. Epidermis and Dermis



Work is needed to improve the efficiency of the printing process and the selection of biopolymers; this would allow enhancement of cell density without cell distortion after printing.

VASCULARITY

One of the biggest complications is the creation of vascular networks in the implantation of tissues to achieve functionality; these networks are important for the delivery of nutrients and the expulsion of sweat or waste and contribute to the formation of complete tissues. The size of the vascular networks is around 100 to 200 µm.[3]

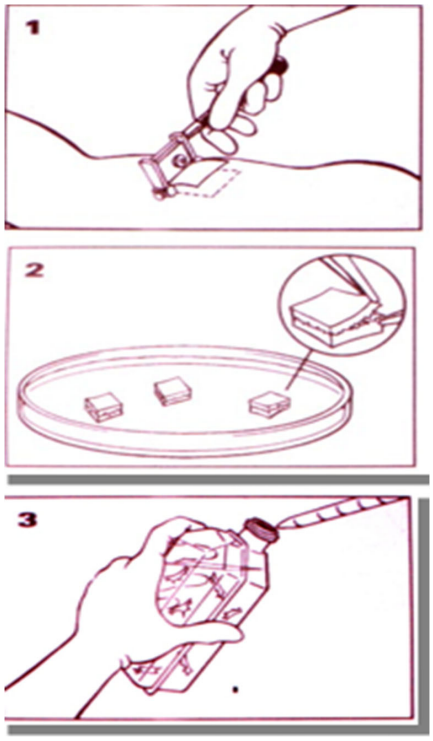
Printing of capillary vessels (vascularity) has limitations in terms of resolution and speed.[3] The capillary vessels must have a dimension of 3 µm, whereas the highest resolution of a laser printer is 20 µm.[3] In addition, printing time is important for cell viability. Scaffold fibrin (dermis) and human microvascular endothelial cells have been used for vascularity.[5]

One option is to perform vascularization live by incorporating angiogenic growth factors and bioink. This can improve the vascularization growth.[3] Vascular networks of synthetic origin[3] can be used as another alternative. Vascular networks that allow the functionality of the implanted tissue are presently under development. One of the techniques is to create sunken channels in the hydrogels through 3D printing.[4]

CLINICAL THREE-DIMENSIONAL BIOPRINTING: PERSONAL TECHNIQUE

Our 3D bioprinter techniques uses live cell cultures, which is a much faster process (see Figure 2, Figure 3, Figure 4, and Figure 5). It allows the porosity of the

FIGURE 3. Biopsy of the Patient



dermis and epidermis to be regulated. Therefore, it is possible to orient the cell according to its size and shape.[6]

Dermis-epidermis cells are first obtained from a patient biopsy (3 × 2 cm). Next, an enzyme is placed to perform dermoepidermal separation. Epidermal cells are thus separated and isolated in a medium enriched with platelets from the patient.[6]

Gel preparation is then carried out with collagen, hyaluronic acid, and chitosan. Cells from the patient's

FIGURE 4. Gel Preparation

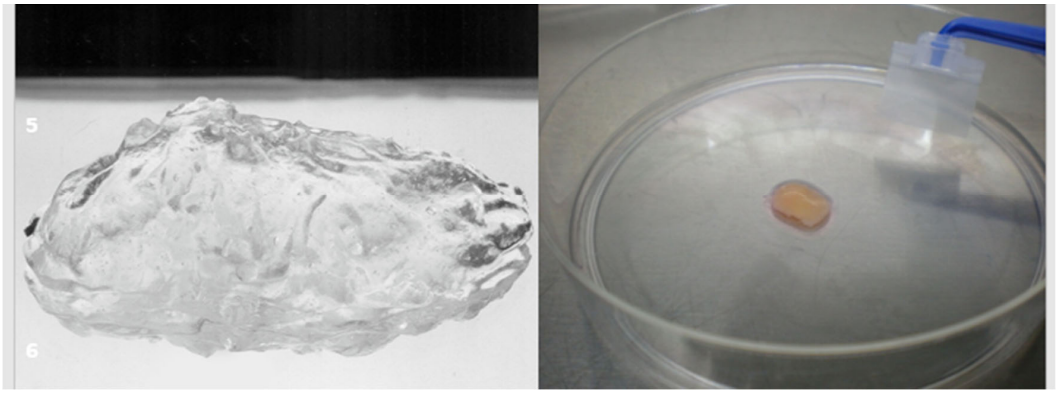
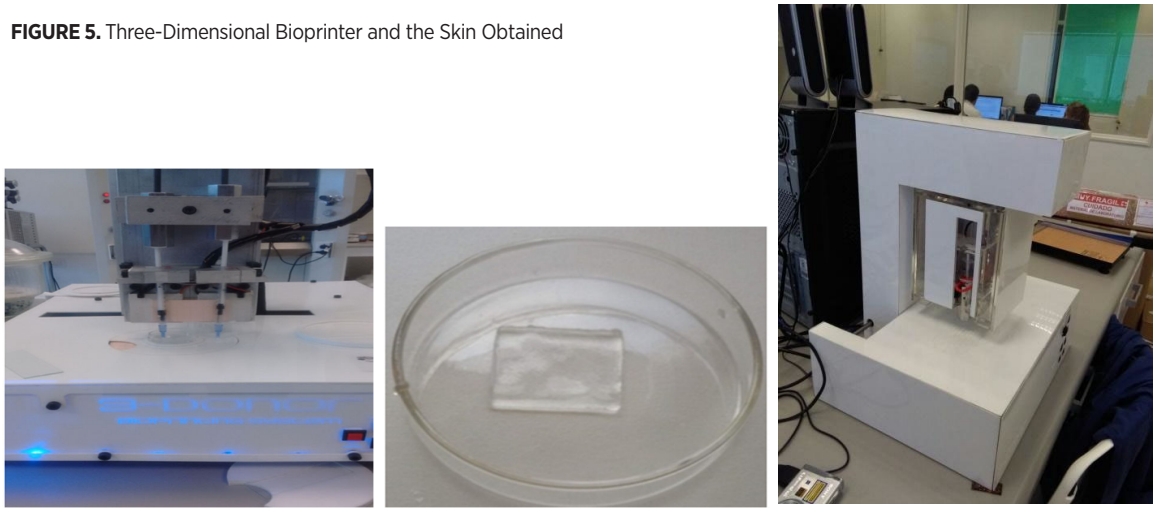


FIGURE 5. Three-Dimensional Bioprinter and the Skin Obtained



platelet-enriched plasma are then placed. The dermis and epidermis are printed with the gel and the cells according to the desired size and thickness and the needs of the patient.[6]

The printer is a sterile flow printer with adjustable temperature that allows rapid drying of the skin sheets. The dermis is constituted of collagen and chitosan and hyaluronic acid. The epidermis is constituted of the same plus epithelial cells and the platelet-rich plasma of the patient. The ink can be improved to maintain the viability and proliferation of the epidermal cells during printing.[6]

CONCLUSIONS AND RECOMMENDATIONS

Three-dimensional printing has many applications in engineering, industry, and medicine. In medicine, the most important application is tissue and organ engineering. Tissue engineering has made major advancements for the manufacture of skin and grafting. It has also contributed to important scientific studies in the evaluation and discovery of drugs, skin disorders, and diseases.[1]

Before 3D bioprinting, conventional methods of tissue engineering were used. These older methods had little spatial relationship between the individual elements (cells) of the desired tissue.[3] With 3D bioprinting, it is now possible to improve both spatial resolution and reproducibility and to obtain optimal conditions for cell incubation and maturation.

Three-dimensional bioprinting allows respective layer-by-layer aggregation of cells and the organization of multiple cell types in a desired structure.[4] Cell culture is performed in vitro (3-4 weeks), allowing growth and maturation to achieve the desired tissue. After these steps, tissue implantation can be performed.

The bioprinting technique consists of the following stages: software development (using computers, image acquisition, and software) for the design of the tissue or organ and the control of cell deposition, printing (cell and hydrogel deposition), and maturation of the tissue or organ (incubation or the use of a bioreactor). This last stage involves the use of biological techniques of cell assembly and fusion or aggregation (use of a reactor).[2] It is also possible to perform implantation of tissue in the skin without an incubation process (clinical bioprinting).

With clinical bioprinting, it is possible to avoid the incubation stage and the maturation time for the tissue (3-4 weeks). With this method, the skin is implanted directly from the printing to the patient.

Among the different bioink components used for bioprinting are synthetic scaffolds, natural scaffolds, and hydrogel scaffolds.[3] Printing techniques include inkjet 3D bioprinting, microextrusion 3D bioprinting, and laser 3D bioprinting. The microextrusion technique is the most used, as it offers cell deposition and spatial resolution.

Three-dimensional bioprinting is of great importance in the field of biomedicine. For example, organ transplantation is one of the most important treatments in medicine for many organ disorders. With limited supply of donors, the biofabrication of organs and tissues can help with this need.[4]

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