AAPS PharmSciTech (2023) 24:191
https://doi.org/10.1208/s12249-023-02643-3

REVIEW ARTICLE

Novel Advances in 3-D Printing Technology in Drug Delivery

New Insights into the Applications of 3D-Printed Biomaterial in Wound Healing and Prosthesis

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Received: 8 June 2023 / Accepted: 23 August 2023

Abstract

Recently three-dimensional bioprinting (3D-bioP) has emerged as a revolutionary technique for numerous biomedical applications. 3D-bioP has facilitated the printing of advanced and complex human organs resulting in satisfactory therapeutic practice. One of the important biomedical applications of 3D-bioP is in tissue engineering, wound healing, and prosthetics. 3D-bioP is basically aimed to restore the natural extracellular matrix of human's damage due to wounds. The relevant search was explored using various scientific database, viz., PubMed, Web of Science, Scopus, and ScienceDirect. The objective of this review is to emphasize interpretations from the pre-executed studies and to assess the worth of employing 3D-bioP in wound healing as well as prosthetics in terms of patient compliance, clinical outcomes, and economic viability. Furthermore, the benefits of applying 3D-bioP in wound healing over traditional methods have been covered along with the biocompatible biomaterials employed as bioinks has been discussion. Additionally, the review expands about the clinical trials in 3D-bioP field, showing promise of biomedical applicability of this technique with growing advancement in recent years.

Keywords bioinks · biomedical · prosthetics · skin regeneration · tissue engineering · wound dressings

Introduction

Pharmaceutical technology is in the effort of doing continuous advancements in the field of drug design in the areas of manufacturing, processes, and improved quality of dosage form. The past few decades have focused on developing patient-compatible and acceptable drug delivery systems and dosage forms that can come up with satisfactory therapeutic outcomes. Growing demands for personalized medicines and devices in combination with technological innovation have proved to be very beneficial and have shown progress in the last few years [1]. Amongst all these advancements, three-dimensional printing (3DP) is a newly emerging technology, which is found to be a versatile tool for manufacturing numerous devices, tissues-organ engineering (prosthetics) and dosage forms [2]. Along with the medical arena, 3DP has a wide application in other disciplines such as aerospace, fashion industry, and construction area [3].

As per the International Standard Organization (ISO), 3DP is defined as "fabrication of materials/objects/devices through the deposition of a material using a print head, nozzle or another printer technology" and is referred as the fastest emerging technologies with an advancement and application rates in leaps and bounds [1]. In this method of additive fabrication, the formulations/medical devices, *etc.* are fabricated employing 3D model data through amalgamation of combining and constructing process materials in layer-by-layer approach [4]. The use of additive manufacturing has brought a paradigm shift in the pharmaceutical industry, moving closer towards the theory of personalized medicine. Personalized medicine is beneficial in terms of declining dose-dependent adverse effect or chances of subtherapeutic levels specifically with the drugs holding narrow therapeutic window and results in satisfactory clinical outcome and patient compliance. Personalized medicine comprises catered dosage forms for unique populations, particularly paediatric, geriatric, or dysphagic patients offering convenience for medication [5].

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Published online: 19 September 2023

The suitable strategy of additive manufacturing is known as rapid prototyping. Speedy prototyping is a necessary feature of the device that is aimed for innovative work region so as to fit with real modern bearings, as well as decreasing time and cost during initial conceptualization. This ensures the reduced chance failure at the later stages.

This technology has several benefits over the conventional technologies, comprising of customization and personalization of dosage forms/formulations (adjusted doses) that can be catered according to individual's need, aptitude to developing complex solid dosage forms having extreme accuracy and precision. Furthermore, it has a great application in modulating release of drug, enhancement of solubility [6].

The present review focuses on the description of significance of 3D-bioP in wound healing and prosthetics along with a brief discussion about various 3D-bioP technologies and the challenge associated with technology as well as bioink development. The review has demonstrated the findings of both *in vitro* cell culture and *in vivo* studies that utilized 3D-bioP employing bioinks for promoting wound healing and 3D-bioP structures for prosthetics. Furthermore, we also provide the current trends and clinical trials that are recently going on utilizing 3D-bioP.

Wound Healing Cascade

Wounds have very common occurrence, and every person has encountered with wound often in their lifetime. There is no definite classification of wounds. However, some of the literature suggests the classification of wounds based on their causes. One of the major causes of wound or injury is mechanical which comprises abrasion or tears of the superficial skin surfaces. Mechanical injuries include abraded, punctured, incised, cut, crush, torn, gunshot, and bite wounds. Another cause of injury is thermal which includes wounds caused by burning and freezing. Other causes include chemical and radiation which could range from skin irritation to necrosis (corrosive chemicals and UV radiation). However, some wounds are classified as acute and chronic (diabetic foot ulcers, pressure ulcers, venous leg ulcers, 3rd-degree and 4th-degree burns, *etc.*). All the above-mentioned wounds whether acute or chronic must need an effective treatment to avoid further complications and severe infections which may lead to serious issues like amputations.

One of the most intricate processes in the human body is wound healing. It involves the coordinated movement of numerous cell types with different functions during the stages which are described below (Fig. 1).

Haemostasis and Inflammation

The inflammatory stage is depicted through haemostasis and inflammation. During the occurrence of wound, the activation of clotting cascade in intrinsic and extrinsic pathways is initiated after the exposure of collagen, triggering the inflammatory phase. During cell injury, a substantial release of thromboxane A2 and prostaglandin occurs causing vasoconstrictions. The clot comprises of collagen, platelets, thrombin, and fibronectin,

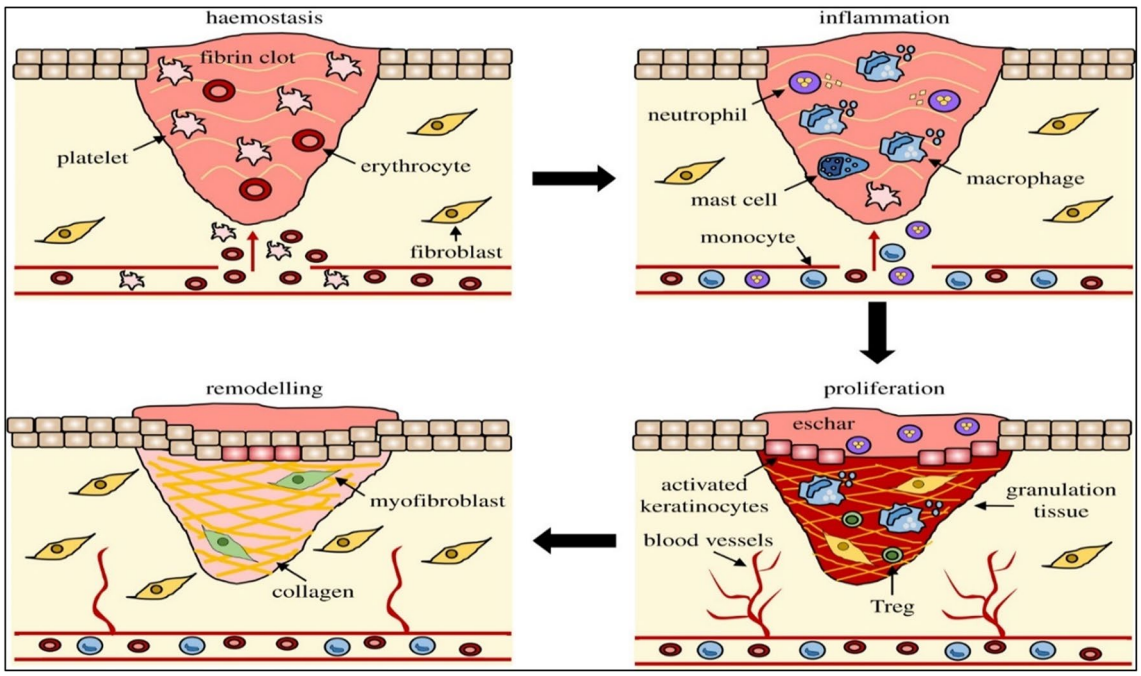

Fig. 1 Wound healing cascade. Reproduced with permission [7]

and all these initiate the inflammatory response ascribing to cytokines and growth factors (Gf) release. The fibrin clot acts as a scaffold for the arrival of cells such as neutrophils, monocytes, fibroblasts, and endothelial cells. It further facilitates in the concentration of cytokines and Gf [8].

Inflammation (Chemotaxis and Activation)

A cellular distress signal is sent immediately after the clot forms, and neutrophils are the initial responders. A facile passage of neutrophils ascribing to vasodilation towards the injured tissue is facilitated through accumulation of inflammatory mediators resulting in prostaglandins generation in the vicinity of blood vessels. Concomitantly, within 46–96 h, the monocytes are drawn from the surrounding tissue resulting in pooling of blood into wound vicinity resulting into macrophages transformation. The inflammatory cell activation, particularly macrophages, is critical. An activated macrophage is required for the cell to enter the proliferative phase. This activated macrophage will facilitate fibroplasia, angiogenesis, and nitric oxide synthesis. Furthermore, neutrophils will invade into the wounded area performing task of clearing bioburden and cellular debris credited to the presence of caustic proteolytic enzymes capable of digesting bacteria and nonviable tissue. This event is followed by invasion of leukocytes and macrophages that appears in the wound [9].

Proliferative Phase

The primary steps in this phase of wound healing are epithelialization, angiogenesis, granulation tissue generation, and collagen deposition. The epithelial cells migrate upward in the normal pattern if the basement membrane remains intact. The epithelial progenitor cells remain intact (in skin appendages), and the normal epidermis layers regenerate in 2 to 3 days. TNF-α–induced angiogenesis is characterized by endothelial cell migration and capillary formation (George et al.2006). Capillary migration into the wound base is essential for appropriate wound healing. Epithelial cells at the skin's boundary commence to proliferate and develop projections in order to reinstate a protective barrier in order to prevent fluid loss and bacterial attack. Epidermal growth factor (EGF) and TGF-α, which are formed by activated platelets and macrophages, stimulate epithelial proliferation and chemotaxis (fibroblasts do not appear to synthesise TGF-α). The final stage is labelled by formation of granulation tissue in which proliferative fibroblasts migrate into the wound site from adjoining tissue, resulting in activation of collagen synthesis and proliferation [10].

Remodelling and Scar Maturation

Subsequent to granulation stage, the synthesis and remodelling of the extracellular matrix have activated and continued for extended periods of time. As the extracellular matrix is constantly remodelled, synthesis and breakdown of collagen simultaneously occur and attain steady state in nearly 21 days subsequent to wounding. Wound contraction occurs as a result of fibroblast interactions with the adjacent extracellular matrix and is affected by cytokines, such as transforming growth factor, platelet-derived growth factor, and basic fibroblast growth factor [11].

Traditional *Versus* Advanced Wound Dressings

Conventional wound dressings are basically designed as a supporting base for the wound and other injuries. The wound dressing comprises of bandages, gauze (sterilized and unsterilized), fibres of natural and synthetic origin such as cotton, cellulose, *etc*. The dressings are fabricated with an aim of imparting absorbent ability that can absorb wound exudates and ideally need not be changed frequently [12]. On the contrary, the available dressings lack substantial absorption capability and inability to prevent further infection resulting of wound exudates and interfere with re-epithelization during peeling of or change in dressing. However, for accelerated wound healing, a dry and clean dressing along with the ability to provide optimum moisture is required. Considering these prerequisites, the modern dressing material has been developed employing a blend of natural and synthetic polymers. The recent development in wound dressing includes hydrogels, films, nanofibers, foam, and hydrocolloids that can address the challenges associated with conventional dressings. These innovative dressings are bestowed with desirable properties required for accelerated wound healing, viz., setting moist environment at the wound site and potentially aids in the healing process along with ability to dynamically monitor the wound conditions [13].

Recent Advances in Wound Healing

Advanced wound dressings have an advantage over conventional in terms of providing moist environment over the wound area along with therapeutic/biological properties of their own. The novel dressings are designed in such a way that they can deliver the medicament or bioactive components at the site of injury as well as provide the required strength and flexibility in designing and are breathable, biocompatible, and atraumatic character fulfilling properties of an ideal dressing that leads to accelerated wound healing even though the dressings are considerably suitable for healing third-degree burns and chronic wounds, often encountered with regulatory issues and economic viability [14, 15]. The development of smart and flexible bandages, biomolecule-loaded dressings, and 3D-printed dressings are

a few of the more recent options that have been designed to promote wound healing. Moreover, recent modifications in wound dressings also include a number of microelectronic sensors for monitoring the environment around the wound in real time and being able to take the necessary actions to aid in the healing process [16]. Recently, 3DP has emerged as a versatile technology for fabrication of smart wound dressings. 3DP provides a platform for fabrication of optimized wound dressing as well as tissue engineering that can encase drugs, biomolecules, growth factors stem cells, etc. over scaffolds/devices/dosage forms, as well as competent to develop prosthetics (tissues-organ engineering) and can be designed as per individual's need. Furthermore, the innovative wound dressing is designed with microelectronic sensors that can monitor stepwise wound healing process, biomarkers/physiochemical regulation, and the presence of bioburden, moisture, *etc.* and stimulate to provide the necessary substrate/action required for accelerated wound healing progress [17].

3D Bioprinting (3D-bioP) Technology

Bioprinting (bioP) is defined as "the positioning of biochemicals, biological materials, and living cells for the generation of bioengineered structures (i.e. additive manufacturing) of biological and biologically relevant materials with the use of computer-aided transfer and build-up processes" [18, 19].

In the recent decades, 3D-bioP technology has been utilized in the development of personalized 3D medicines, layer-by-layer cell-laden bioinks, prosthetics, tissue engineering, drug screening, toxicology, bioP, and medical devices. Moreover, 3DP wound dressings have shown significant success in accelerated wound healing, tissue/organ printing, and skin regeneration/repair [20]. The 3D-bioP technology holds remarkable advantages in numerous aspect such as the simulation/similarity and compatibility of 3DP organs/cells, *etc.* in terms of size and internal structure with biological tissues, software-facilitated flexible designing of targeted models, and ability to fabricate the major and minor components of tissue/organ and even cytoplasmic matrix [21] and growth factors [22–24].

There are some special requirements in 3D-bioP that should be addressed and monitored for the success of this technique. One of the considerations is related to the selection of biomaterials. Since bioinks or living cells are used for bioprinting, therefore, the selection of biocompatible materials does not hamper the function and viability of the cells is crucial. Apart from the material selection, the other crucial parameters which require special attention comprise optimizing induced mechanical stress during the process of 3D-bioP of the structures. The other important consideration includes fulfilling the nutritional requirements necessary for cell development during the process as well as post-process ensuring cell survival and cell viability. The employment of suitable bioinks should be ensured that can monitor and maintain the viability and proliferation process of printed constructs. In process and post-process factors that can influence the final bioP product also include bioprinter design and resolution, vascularization, long-term stability and functionality, and some regulatory and ethical considerations [25].

In addition to the numerous benefits of this technique, a number of challenges must be overcome for its development and ubiquitous application. Cell damage is the main obstacle that can be ascribed to the conditions of bioP, which include mechanical shear, temperature change, and pH change. As a result, the viability of the cell could be lost both during and after bioP. Therefore, the selection of types of cells that can survive the printing process is also a constraint. Integrating functional vascularization into bioprinted tissues or structures is another significant hurdle to overcome. Since the cell and tissue architecture and ECM composition of native tissue are extremely complex, there is a possibility that bioprinted tissue may lack complexity. Other obstacles to the widespread use of bioprinting include cost, scalability, regulatory restrictions, and ethical issues. But with improvements in bioink formulation, printing methods, biomaterial development, and tissue maturation processes, scientists and engineers are continuously trying to address these difficulties. To solve these issues and maximize the use of bioP in regenerative medicine, more research, collaboration, and technological advancements are required [26].

3D-Printed Bioinks for Wound Healing

The 3D-bioP has made progression and has become technically innovative and sound and economically viable, reliable, and convenient handling with a wide range of 3D printers. Furthermore, they can be designed according to the need and modification which can be done in terms of number of extruders, speed, temperature range, desirable thickness of printed material, *etc*. However, the most challenging task is to develop a bioink specifically loaded with live cells, and this adds up an extra constrain for fabrication of 3D printers, as it is very difficult to assure the survival of loaded cell in the course of the bioprinting [27]. Conceivably, the printing of scaffold followed by seeding of cells on its surface could be a probable solution of the aforementioned challenge. Bioinks are the prerequisite for 3D-bioP that involves construction of complex matrix comprising of biological materials such as cells and/or crosslinkers and some biomaterial that facilitate cells functionality when administered *in vivo*. Furthermore, the bioink should possess specific characteristics such as high biocompatibility facilitating cell growth and mechanical strength and commit shape firmness subsequent to printing and ensure survival of cells before reaching the maturation stages and succeeding time [28]. However,

the challenges encountered with bioinks are maintenance of shape, functional integrity, viscosity, degree of crosslinking, gelation time, and influence of shear during fabrication. Furthermore, mechanical interference is offered through printing parameters such as nozzle diameter and temperature, flow rate, and printing duration [29].

Additionally, the selection of cells is a very critical parameter for restraining chances of immune rejection. Bioinks are categorized into natural and synthetic domain and are widely employed in tissue engineering, scaffolds for wound healing and prosthetics [30, 31].

Natural-Based Bioinks

Bioinks of natural origin are bestowed with non-toxicity, biocompatibility, biodegradability, mechanical strength, and non-immunogenic attributes, along with the capability of maintain the optimum moisture content required for wound healing that makes them favourable for the usage in 3DP [32, 33]. Furthermore, the important prerequisite of a successful bioink preserves functionality of cells so as to facilitate ECM stimulation to simulate the desired skin/tissue microenvironment [34]. Various literatures have reported the utilization of sericin, fibrin, gelatin, hyaluronic acid, alginate, chitosan, collagen, agarose, cellulose, and decellularized extracellular matrix (dECM) for developing natural bioinks for fabricate bioinks. Recently, an amalgamation of one or two biomaterials such as gelatin and collagen has been employed in the development of bioinks to improve physiochemical and mechanical strength as well as decreased immunogenic response as these components naturally exist in human body and are biocompatible and hold printing fidelity [35].

Synthetic Polymer-Based Bioinks

The literatures have reported the utilization of synthetic polymers for 3D-bioP. The popular polymers comprise of pluronic acid, polyethylene glycol (polyethylene (glycol) diacrylate (PEGDA) and poly (ethylene glycol) methacrylate/dimethacrylate), poly(l-lactic) acid (PLA), polyvinylpyrrolidone, PLGA (poly(lactic-co-glycolic) acid), polycaprolactone, etc. The most significant advantage of utilizing synthetic polymers is the flexibility to cater the degradation and mechanical attributes in line up with obligation of the targeted tissues and organs. However, their use is limited owing to numerous constraints such as high melting point with respect to body use of solvents and poor encapsulation efficiency. Furthermore, these polymers also deficient of sites for cellular specificity and biological indications represented through ECM that facilitates cellular proliferation and differentiation. However, these drawback can be addressed through functionalization of synthetic bioinks [36].

Methods of 3D-bioP

On a broad basis, 3D-bioP can be categorized into four types, viz., inkjet-based bioP, stereolithography, extrusion-based bioP, and laser-assisted bioP.

Inkjet-Based bioP

The inkjet-based printing technology (Fig. 2) is applied widely in tissue engineering but encountered with a limitation of cell mortality during printing owing to rapid drying after coming out on the substrate [37]. The abovementioned limitation was addressed through encasing cell into an extensively hydrated polymer in form of cell-loaded hydrogels. The merits include economic viability, great printing speed, and easy availability. Conversely, poor entrapment efficiency, low droplet directivity, and incapability to squeeze high-viscosity stuffs are some of the demerits connected with this technique [38].

Extrusion-Based bioP

Extrusion-based bioP or pressure-assisted bioP as bioink dispensing is facilitated through pneumatic or mechanical-driven pressure air pump that provides capability to work with extreme cell density (Fig. 3) [39]. On the contrary, it has some demerits such as compromised resolution when compared with other techniques that limits its application for soft tissues. Also, the pressure during extrusion may alter the morphology and functionality of the cells [37].

Laser-Assisted bioP

Laser-assisted bioP is performed through laser for bioP (Fig. 4). In this technique, the donor layer holds a ribbon-like structure at the base along with an energy absorbing layer at the top, while bottom layer consists of bioink solution. The mechanism involves stimulating a portion of absorbing layer resulting in generating high-pressure bubble at bioink layer interface causing bioink propulsion and the droplet of bioink get crosslinked to substrate. The major advantage of this technique is high cell viability as it is a noncontact, nozzle-free printing technique and does not induce mechanical stress over cells as well as high resolution. However, it is an expensive technique thus is less often used [41, 42].

Stereolithography

Stereolithography is a nozzle-free bioP technology in which a photo-sensitive polymeric liquid formulation is solidified subsequent to illumination. The micromirror arrays are utilized to optimize the light intensity required for polymerization of formulation. Stereolithography is commonly employed to develop photo crosslinked

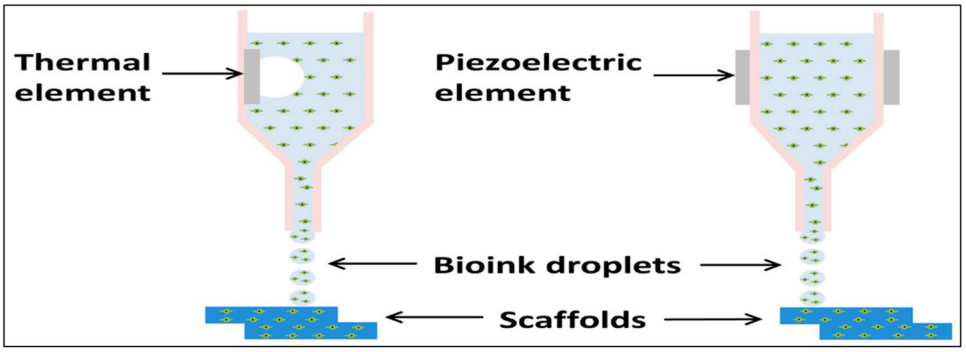

Fig. 2 Inkjet-based bioP. Reproduced with permission [37]

polymers having numerous applications. This technique is bestowed with ultimate fabrication accuracy and is utilized to fabricate hydrogels with variable thickness and differential layers (applied a s wound dressings). However, it has some demerits such as limited availability of biocompatible and biodegradable polymers, toxic residual reagents, and incompetence to eliminate supporting structure absolutely and failure to produce horizontal gradients constructs [42].

Recent Advances of 3D-bioP in Wound Healing

Numerous pieces of literature have suggested the application of 3DP in wound healing. The 3DP has proved its potential in wound healing along with low cytotoxicity and structural similarity to extracellular matrices. Considering the merits of 3DP, Fayyazbakhsh *et al.* uses extrusion-based 3D printing technology for the fabrication 3DP hydrogel wound dressings for deep-partial thickness burn wounds. The cell laden dressings bioprinted using 75% gelatin and 25% alginate showed best mechanical properties as well as healing aspects in case of deep burns [43]. Another study by Zhao *et al.* fabricated 3D-printed artificial skin patches with cationic conjugated poly(phenylene vinylene) derivative (PPV) and gelatin/alginate/hyaluronic acid for prevention of infection and augmentation of wound. Studies showed that patches with PPV had excellent activity against *S. aureus* as well as it assists the regeneration of tissue [44]. Another group of researchers, Brites *et al.*, fabricated biocompatible and antibacterial 3D-printed patches of Manuka-gelatin by using extrusion-based printing technology for wound healing application. Results showed that patches formed hold antibacterial activity against both gram-positive and gram-negative bacteria that are responsible for causing wound infections. Furthermore, studies also revealed that patches enhanced the proliferation of dermal fibroblasts and also promoted angiogenesis [45].

In another study, Guan *et al.* fabricated angiogenic 3D-bioP peptide coupling patches for wound healing. The patches formed were combination of pro-angiogenic peptide, gelatin methacryloyl (GelMA), and hyaluronic acid methacryloyl (HAMA). Results of the study revealed that the use of 3D-bioprinted patches showed extended release of pro-angiogenic peptide along with improved angiogenesis and tissue repair [46].

Tsegay *et al.* fabricated smart 3DP auxetic hydrogel wound dressing by using digital light processing printer. This colorimetric sensor was incorporated for monitoring of pH levels as well as status of wound healing. The developed dressing was able to prevent the exacerbation of diabetic wound [47].

Xu *et al.* fabricated nanocellulose conjugated with gelatine methacrylate scaffolds for wound healing using customized extrusion-based 3D printer [20]. The scaffolds were printed utilizing 1% w/v 2,2,6,6-tetramethylpiperidine-1-oxyl radical-oxidized carbon nanofiber (CNF) and 1% w/v gelatin methacrylate. The prepared scaffolds exhibited good mechanical strength of 2.5 to 5kPa and showed biocompatibility with

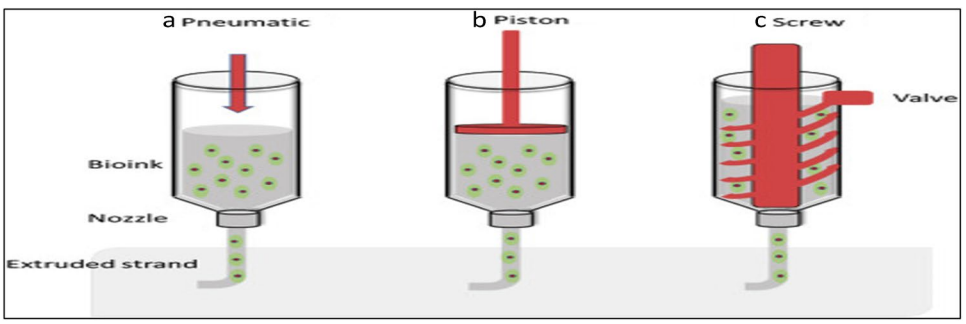

Fig. 3 Extrusion-based bioP. **a** Pneumatic, **b** piston-driven, **c** screw-driven mechanical extrusion. Reproduced with permission [40]

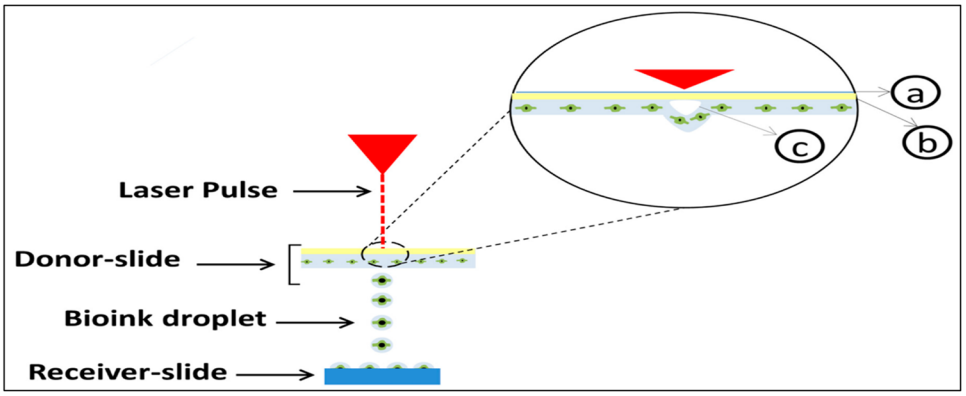

Fig. 4 Laser-assisted bioP. Reproduced with permission [37]

3T3 fibroblasts cells. The 3DP scaffolds showed significantly enhanced proliferation of 3T3 fibroblast cells, i.e. twofold when compared with cellulose nanofibrils, suggesting promising formulation for accelerated wound healing and soft tissue regeneration. Similarly, Teoh *et al.* investigated the versatility of chitosan methacrylate in the fabrication of 3DP wound dressings for burn treatment (Fig. 5). They fabricated the wound dressing using BioX 3D printer (pneumatically driven extrusion-based bioprinter). The study revealed that chitosan methacrylate is printable, biodegradable, and biocompatible. Furthermore, the addition of levofloxacin, an antimicrobial agent, improves its antimicrobial capabilities significantly [48]. Another study by Wu *et al.* demonstrated the silver-ethylene conjugation and 3DP to fabricate antibacterial super porous polyacrylamide/hydroxypropyl methylcellulose hydrogel dressings. Silver-ethylene interaction exhibited a crucial role in promoting the formation, dispersion, and crosslinking of silver nanoparticles with the matrix of hydrogel, as well as the crosslinking of the polyacrylamide networks. The material showed good water uptake capacities, i.e. 14 times its dead weight [49].

Another group of researchers, Maver *et al.*, in their investigation fabricated a pain-relieving wound dressing by combining 3DP and electrospinning technique. In their study, they incorporated NSAID and local anaesthetic (diclofenac sodium and lidocaine) into a wound dressing that was a combination of 3D bioprinted carboxymethyl cellulose-based scaffold (prepared by piston-driven extrusion-based bioprinter) and electrospun CMC-based nano-mesh. The result of the studies revealed that the fabricated product was biocompatible and effective pain-relieving wound healing dressing [50].

Hafezi *et al.* fabricated crosslinked chitosan-based film matrices using inkjet 3D bioprinting technology and using genipin as a crosslinker, as well as glycerol and polyethylene glycol (PEG) as plasticizers. PEG600 3DP films with a polymer, plasticizer ratio of 1:1, were found to have appropriate flexibility. Furthermore, MTT assay (human skin fibroblast cell lines) confirmed 90% of cells viability subsequent to 48 h of exposure witnessing non-toxic attribute of the 3DP films. This gives an auxiliary proof that 3DP can be promising for fabricating dressing for chronic wound healing applications [51].

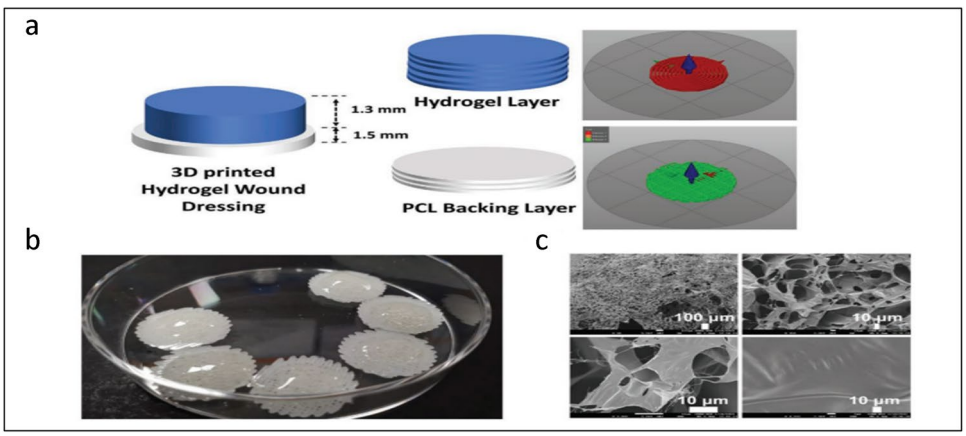

Fig. 5 **a** A diagram of the hydrogel wound dressing design that was employed in the research. The wound dressing is made of a 3DP poly(caprolactone) wafer that serves as both the backing layer and the substrate for the printing of chitosan methacrylate. **b** Digital representations of 3D-printed chitosan-methacrylate dressings. **c** Surface morphology executed through FESEM at various magnifications of 3DP chitosan methacrylate made with 4% (w/v) chitosan methacrylate. Reproduced with permission [48]

Muwaffak *et al.* fabricated antibacterial wound dressing using hot melt extrusion method. Metals having antimicrobial properties such as zinc, copper, and silver were used to make 3D-printed filaments, which were then included into a polymer matrix of polycaprolactone. The patient's nose and ears were scanned in 3D to create 3D models, which were then utilized to create a wound dressing that was tailored to the patient's unique shape and size (Fig. 6). 3DP was used to optimize size and shape of dressing. It was also found that all metal dressings exhibited fast release for initial 24 h followed by delayed release over 72 h. Using a thermal activity monitor device, the antibacterial efficacy of the wound dressings was determined, demonstrating that silver and copper wound dressings were the most effective [52]. Kanjou *et al.* fabricated scaffolds that used modified bacterial cellulose (change in fermentation medium) with hyaluronic acid chondroitin sulphate. Furthermore, it was crosslinked with sodium alginate and calcium chloride that produced a desirable 3D structure. The produced scaffolds were found to be effective in healing of diabetic ulcers [53].

Alizadehgiashi *et al.* fabricated a multifunctional 3D-printed wound dressing using extrusion-based technology. They designed a mesh type hydrogel for the purpose of personalized wound cure. In formulation, the host hydrogel was made up of cellulose nanocrystals and chitosan methacrylamide, whereas the distinct filament in hydrogel wound dressing was loaded with therapeutic agents such as antibiotics and silver nitrate to prevent bacterial load during wound healing process. Further studies showed that mesh type design allowed the passive differential release of biologically active agents and improved the wound healing [54].

Ilhan *et al.* used extrusion-based bioprinter for the fabrication of a scaffold containing extract of *Satureja cuneifolia* combined with sodium alginate/polyethylene glycol (PEG) for the potential treatment of diabetic ulcer (Fig. 7). Scaffolds were prepared by adding different concentrations of PEG to 9% w/v sodium alginate. The studies revealed that scaffolds have excellent antibacterial effect against gram-positive bacteria and have a great potential for the infectious and diabetic wound healing [55]. Wang *et al.* created unique bionic hydrogels possessing antimicrobial and free radical scavenging properties that efficiently treat the wound by reducing inflammation and speeding wound healing (Fig. 8). Glycidyl methacrylate-modified carboxymethyl cellulose (CMC) and-polylysine (PL) were added to the CMC/PL hydrogels and were customized using a 3D printer and UV light polymerization. Studies revealed that CMC/PL hydrogels showed up to 95% inhibitory effect on both species of *Escherichia* and *Staphylococcus* as well as attribute to protect the fibroblasts from damage. Moreover, on comparison with the marketed product (Tegaderm™ film), fabricated hydrogels exhibited elevated capability to increase the expression of VEGF and CD31 that was responsible for accelerated tissue regeneration and promoting wound healing [56]. Similarly, another group of researchers, Hu *et al.*, develop a 3D scaffold dressing using extrusion-based cryogenic 3DP technology. In this, a scaffold possessing combination of decellularized small intestine submucosa, mesoporous, and bioactive glass was developed to allow for prolonged release of bioactive exosomes. The prepared hydrogel scaffolds have a good 3D structure with appropriate porosity, biocompatibility, and haemostasis ability, which aids in the accelerated proliferation, migration, and angiogenesis of human umbilical vein endothelial cells. Furthermore, the studies showed that hydrogel scaffolds increased the blood circulation in wounds and speed up the angiogenesis progression in diabetic wound [57].

Nuutila *et al.* established the efficacy of growth factor-eluting adhesive scaffolds printed *in vivo* for the managing full-thickness wounds. The printing of gelatin-methacrylate hydrogel encasing vascular endothelial growth factor was done through customized portable printer. The *in situ* hydrogel crosslinking resulted in strong scaffold adherence and allowed printing on curved wet tissues surfaces with no use of sutures. Furthermore, histological studies showed that administration of VEGF eluting GelMA scaffolds had good adherent properties and it also improves the quality of healing [58].

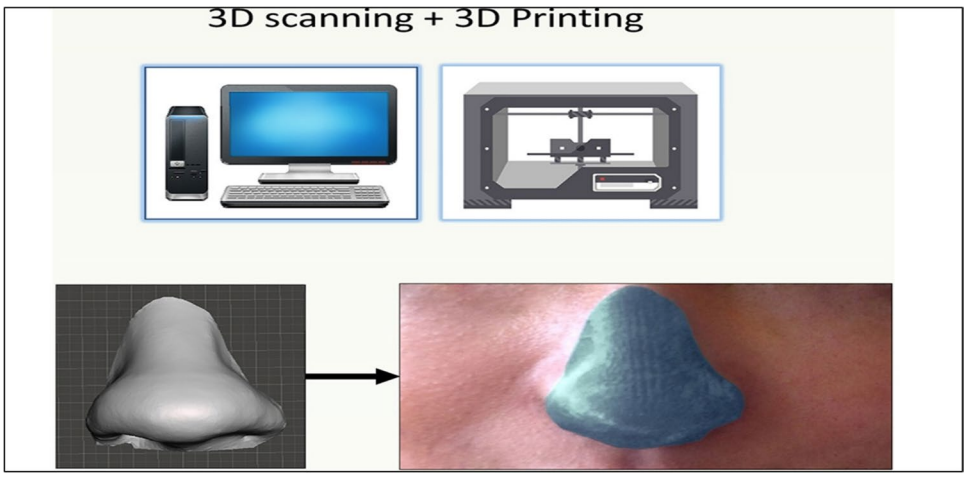

Fig. 6 An outline of the work that was done by Muwaffak *et al.* Reproduced with permission [52]

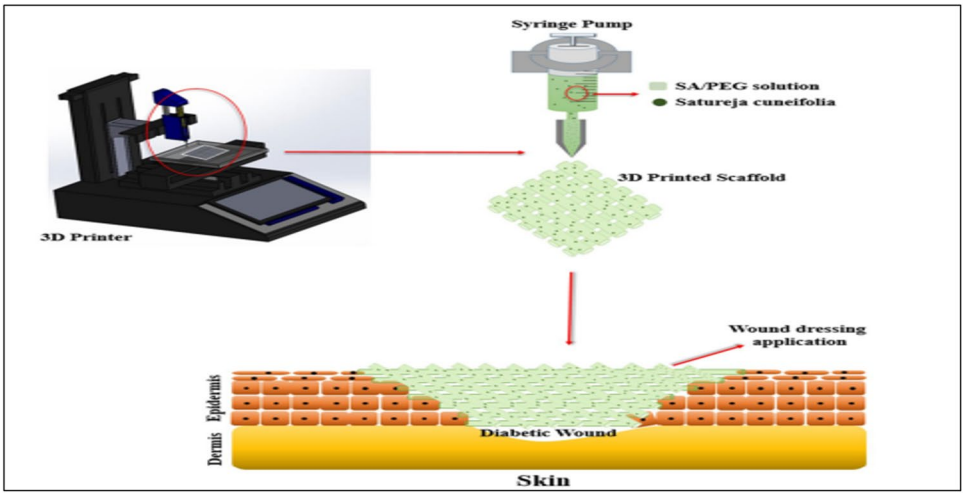

Fig. 7 An outline of the work that was done by Ilhan *et al*. Reproduced with permission [55]

Although extensive works have been executed in the development of wound dressings employing 3D-bioP, it has few limitations that compromise its application in clinical settings and requires further verification developing more suitable and stable bioink. These includes long-term efficacy in preclinical and clinical settings, challenges encountered in regulatory approval, immunogenic-toxicity, immune rejection, and variable cell viability [57].

3D-bioP in Prosthesis

3DP has witnessed to be a helpful technique in the fabrication of prosthesis as it has played a major role in improving the quality of life of patients that have lost any of the body due to certain diseases, birth defects, or trauma. Luo *et al.* provided an optimization method for light weight design of mandibular scaffold based on the concept of uniform stress distribution and used novel metallic thin-film coating and high-resolution 3DP of micro-lattice, titanium film-coated 3D-printed PLA composite lattice, since PLA is a biological material with good toughness and strength and non-toxic that has been widely used in medical field , and the titanium films can both improve the compressive strength of PLA lattice and minimize bacterial adhesion [59]. Wen *et al.* reconstructed damaged tumour wall, as customized titanium alloy chest wall utilizing 3DP. For the fabrication of chest wall, a helical CT data was used, and then the customized designing was done using the computer-aided drug design (CAD); later on, the mechanical properties of the fabricated

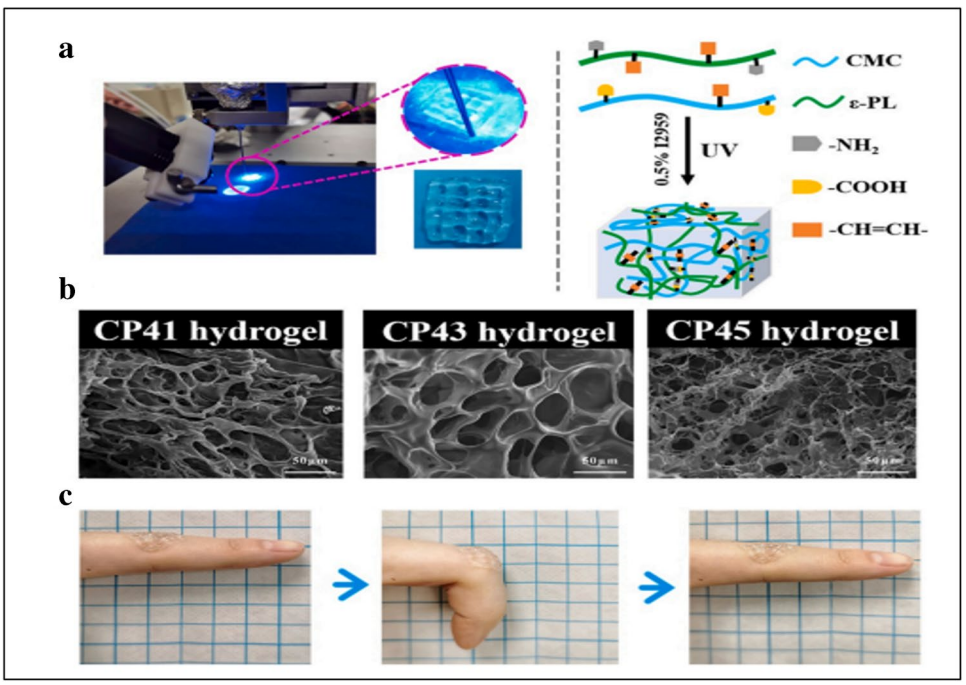

Fig. 8 An outline of the work that was done by Wang *et al*. **a** 3D-printed schematic illustrations of CP hydrogels. **b** Surface morphology through SEM of the prepared hydrogels. **c** A photo of the human finger with optimized hydrogel applied to it. Reproduced with permission [56]

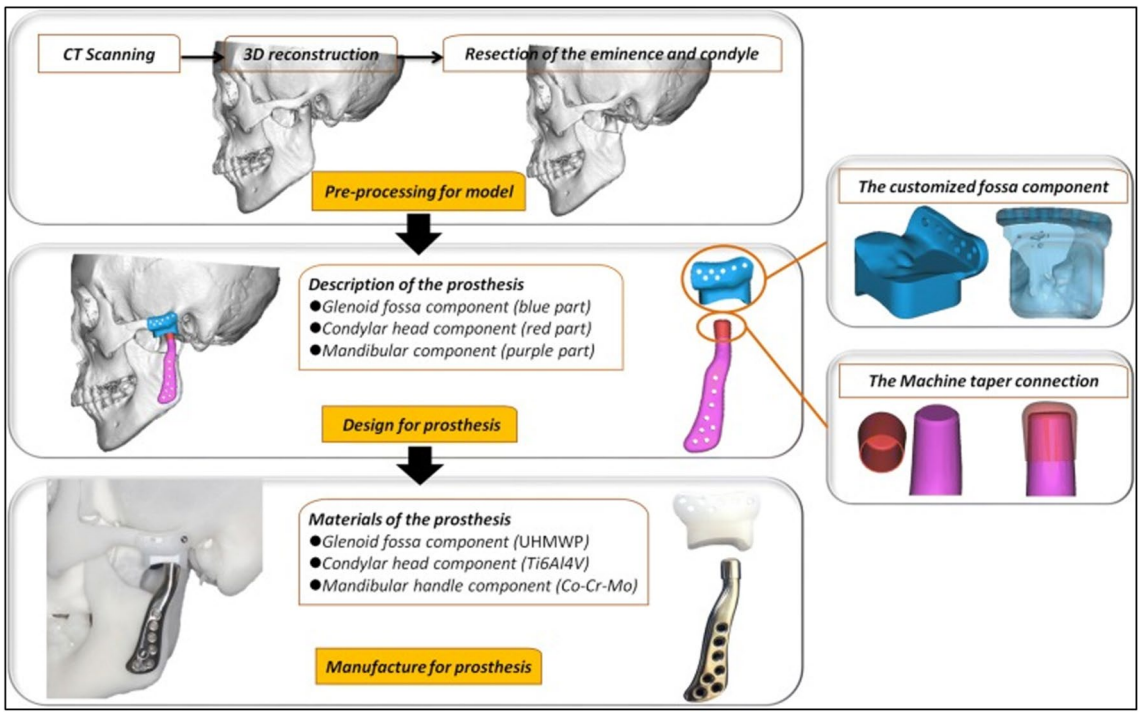

Fig. 9 The steps taken to create the new TMJ prosthesis, from preliminary work on the craniomaxillofacial model to the prosthesis's design and eventual production. Reproduced with permission [63]

prosthesis were investigated using ANSYS software. The printed chest wall was successfully installed, and there was no rejection or infection reported [60]. Zhang *et al.* performed the reconstruction of thyroid cartilage using 3DP along with the parametric modelling method. Fabrication of the cartilage was done by the selective laser melting method using the scan of the affected sites. Ti6Al4V alloy was used for the construction due to its high specific strength and good biocompatibility [61]. Another remarkable facial restoration was done by Ruiters *et al.*, who fabricated the 3D-printed ocular prosthesis as a restoration for eye blindness resulted due to the congenital cataract, glaucoma, and corneal melting. The impression of the patient's left eye was taken by the help of CT scan, and the designing was done using the CAD and was successfully fitted into the patient's eye [62].

Another group of researchers (Zheng *et al.*) fabricated the 3D-printed temporomandibular joint (TMJ) prosthesis for the patients with end-stage TMJ osteoarthrosis (Fig. 9). The CT scans obtained from patient's 3DP reconstruction models were prepared with customized design of the fossa component with polyethylene (ultra-high m.w) and the connection device between the condylar head and mandibular handle components (Ti6Al4 V alloy). The study was conducted on 12 patients, and there were no complaints of infection or loosening of prosthesis [63]. Similarly, Peña *et al.* have done cost-effective cranioplasty using a 3D digital printing model on two patients who presented with cranial abnormalities. CT scan was used to develop and produce a prosthetic model. Both the CT scan data and the prosthesis were sent to a 3D printer to create a physical model with poly-lactic acid, which is further utilized as preliminary design to fabricate the final personalized prosthesis in polymethyl methacrylate. The fabricated parts were put in place surgically by cranioplasties. There was no rejection or infection reported in duration of 6-month follow-up [64]. In another case study, Abdulameer *et al.* fabricated the nasal prosthesis for the restoration of cosmetic look of the patient. With the help of CT scan, they prepared a 3D-printed model and invested into mould. Maxillofacial silicone elastomer was chosen to reproduce the qualities and texture of the skin. The fabricated prosthesis was inserted into the defected nasal area and was retained at site through medical adhesive. Hence, the 3D-printed nasal prosthesis helped the patient to overcome the defect [65]. Another group of researchers, Fuentes-Gonzalez *et al.*, manufactured an EEG-based 3D-printed prosthetic arm for a patient. All of the prosthetic components were 3D-printed with exception of hinges, which were made of flexible material, and the others were made of polylactide. Afterwards, the 3D-printed prosthetic arm was fixed into the patient's stump, and it was observed that the patient was capable to perform the movements of opening and closing of hands [66].

Roos *et al*. corrected the midfacial defect of a patient resulted due to subtotal maxillectomy for removal of

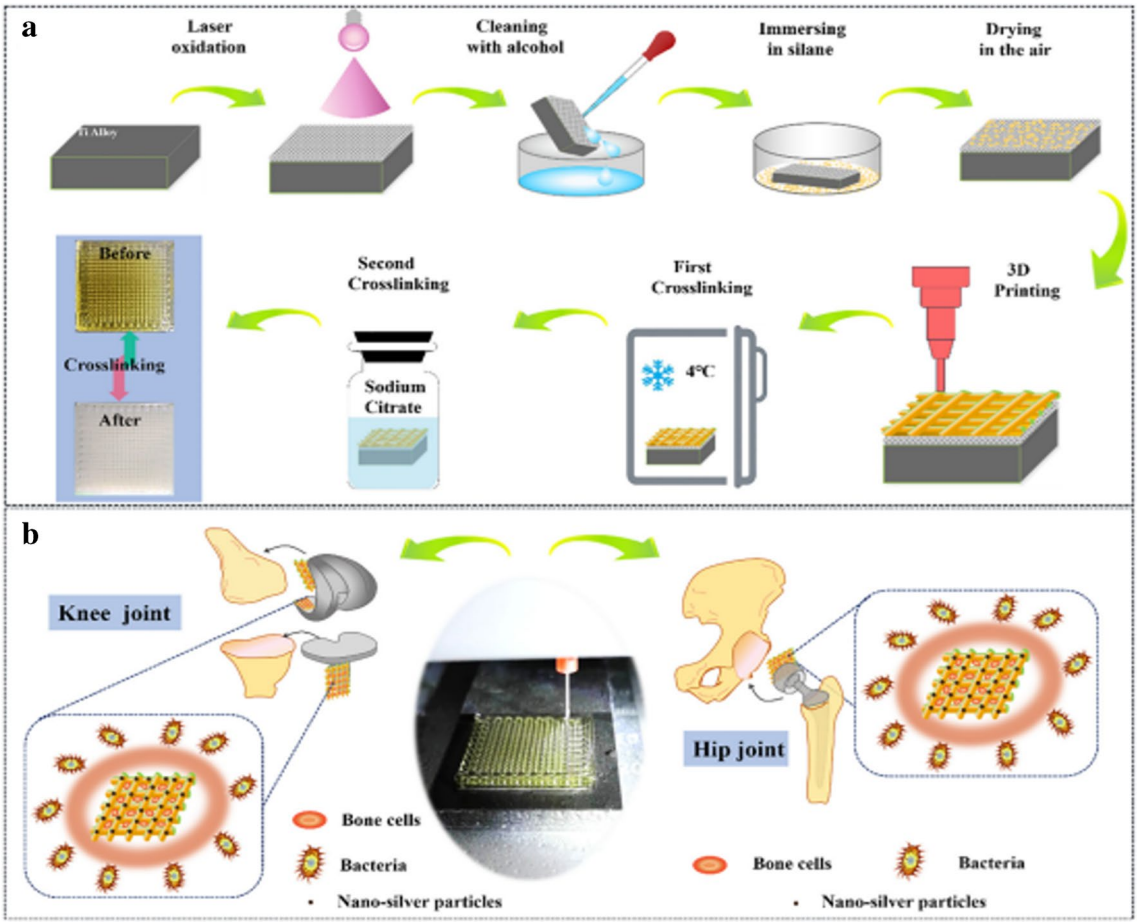

Fig. 10 Diagrammatic representation of the 3DP chitosan-gelatin antimicrobial hydrogel coating production and antimicrobial application processes. **a** The procedure for making the CS-GT hydrogel coating. **b** A biological fixation interface for hip and knee prostheses using 3D-printed CS-GT-nAg antibacterial hydrogel coating. Reproduced with permission [68]

myoepithelial carcinoma of the hard palate. Titanium prosthesis was fabricated by using direct metal laser sintering 3DP technique. Later on, maxillary denture was fixed onto titanium frame. The prosthetic proved its success both in terms of cosmetic and functionality [67]. Wu *et al*. fabricated antimicrobial hydrogel as a biological coating as fixing interface of the artificial joint prosthesis (Fig. 10). 3DP technology was used to print a chitosan-gelatin (bioink) hydrogel covering with reticulated porous structure on a titanium alloy substrate. The experimental results showed enhanced antimicrobial effect of hydrogel after coating with nano-silver against *Escherichia* and *Staphylococcus* species. Hence, the 3D-printed hydrogel was proved to be an excellent biological fixation interface in artificial joint replacement [68]. Nagrath *et al.* fabricated infection neutralizing prosthesis in dentistry. They repurposed polyethyl methacrylate for 3DP as well as tissue surface functionalization with modified release polycaprolactone microspheres encapsulating amphotericin-B for anti-fungal therapy. The findings show that 3D-printed dentures had better mechanical qualities comparable to traditional fabrication methods. In a biomass study, the PCL-PMMA surface was also able to release the medication over long periods of time and actively inhibit *Candida albicans* colonization [69].

However, to address the limitations associated with 3D-bioP a holistic research approach is perquisite involving interdisciplinary collaborations between the clinicians, engineers and formulation scientists. This may result in development of promising 3D-bioP techniques, improved biomaterial for the same having long-term stability, superior biomechanical functionality suitable to pass the clinical trial and finally clinical usage.

Clinical Trials

There are numerous clinical trials which are reported with respect to 3D-bioP for wound healing and prosthesis, some of which are given in Table I.

Table I Clinical Trials Focused on 3D-Printed Wound Healing Material

Title	Patients involved	Period of study	Conclusion	Identifier No.
3D-printed microporous prosthesis for treatment of large metaphyseal segmental femoral bone defect	5	1 year	Personalized 3D-printed microporous prosthesis combined with intramedullary nail exhibited a promising substitute for treating metaphyseal segmental irregular-shaped femoral bone defect, specifically with massive juxta-articular bone loss	[70]
Clinical study of 3DP personalized prosthesis for bone defect consequent to pelvic tumour resection	20	2 years	Results showed that 3DP personalized prostheses for pelvic tumour resection, repair, and reconstruction exhibited high accuracy in tumour resections and matching in prosthesis-patient, declined surgical trauma, and promoting the functional recovery of patients	[71]
3D-printed ankle foot orthoses for stroke patients	12	1 year	Personalized 3D-printed ankle-foot orthoses improved the gait performance better than traditional devices	NCT03965715 [72]
3DP personalized titanium plate in neck and head	45	2 years	3DP of personalized surgical titanium plate is a promising option for head and neck reconstruction when compared with conventional titanium plates. 3DP plates ease and simplify surgical procedures with great precision and accuracy for jaw reconstruction	[73]
Personalized 3DP of titanium plates for jaw surgery patient specific	96	5 years	Three-dimensional personalized titanium plates for jaw helped improvising the reconstruction surgery as compared to heavy titanium plates	NCT03057223 [74]
Assessment of back braces for the spinal deformity	10	2 years	Comparative studies between traditional and 3D-printed back braces were carried out for treatment of adolescent idiopathic scoliosis and osteogenesis imperfecta	NCT04282408 [75, 76]
3DP technology in small pulmonary nodule localization	200	2 years	Enhanced localization accuracy of small lung nodule through 3DP navigational template when compared conventional CT-guided method	NCT02952261 [16]
Comparative clinical study of personalized shoulder, knee, and ankle brace	40	3 years	Study showed that customized shoulder, knee, and ankle brace were better than the conventional braces	NCT04936412 [77]
Advancing prosthetic care in lower limb amputation patients	12	3 years	3D model of the participant's residual limb will be created as base for fabricating 3DP sockets	NCT03517774 [78]
3DP in anatomical lung segmentectomies	34	1 year	Virtual 3D reconstruction of the patient's pulmonary anatomy by using software	NCT05695404 [79]
Assessing the biting force and chewing efficacy of full dentures	27	10 months	The biting and chewing efficiency of 3DP complete dentures were better than that of conventional dentures	NCT04793503 [80]
Clinical use of custom-designed 3D-printed implants for bone defect repair	3000	4 years	Randomized interventional study was conducted on patients. Implants were printed using 3D printing technology and were implanted in bone defect	[81] NCT03166917
Development of a naso-alveolar moulding (NAM) appliance for cleft lip and palate (CLP) using a digital MRI of the face	01	2 months	In this study, a 3D-printed naso-alveolar moulding (NAM) appliance was produced using a face MRI scan, and the NAM's fit in infants born with cleft lip and/or palate was evaluated	NCT04369638 [82]

Table I (continued)

Title	Patients involved	Period of study	Conclusion	Identifier No.
The impact of custom insoles on pain and biomechanics in patients with functional flatfoot	12	3 months	A comparative study was performed to study the impact of 3D printing personalized insoles using various thermoplastic materials on the foot function and biomechanics of functional flatfoot patients	NCT04381039 [74]
A comparison of 3D-printed rigid bolus and silicone bolus for the treatment of skin tumours	20	3 months	In this study, two varieties of 3D-printed skin bolus (stiff and flexible) were compared in order to improve the treatment of skin tumours/cancers. The purpose is to ascertain whether one type of bolus offers a better fit and subsequently radiation plan, the practicality of each type of bolus, and patient-reported feedback	NCT04176900 [75]

Conclusion

Chronic wounds such as diabetic wounds, burns, and trauma have poor and slow healing process and keep a clinical and economic burden on patients and healthcare system globally. Recent years have witnessed tissue engineering and 3DP technology as emerging tool to address the complications associated with wound therapy. The advanced 3DP technology has resulted in the development of processes that are easy, efficient, and economically viable and have led to patient satisfaction and better clinical outcome. 3D-bioP has emerged as the most promising technology in the fabrication of cell-laden scaffolds for wound repairing and tissue regeneration tissues, as well as the fabrication of entire artificial tissue holding proper functionality. Also, it enables the construction of substitutes, such as the skin, bone, vessels, ear, and nose, using human cells. As discussed in the present review, a wide range of 3D-bioP techniques can be catered to form complex geometrical structure simulated to organ/body part that is bestowed with structural integrity and can accelerate healing time as well. Furthermore, the employment of 3D-bioP in wound healing and skin regeneration has concrete direction for the enhancement of advanced therapeutical approaches. Conversely, natural or synthetic biomaterials with excellent biocompatibility and antimicrobial activity have been effectively used for 3D-bioP and are utilized for wound healing applications. 3D-bioP enables the fabrication of homogeneously distributed cell scaffolds. These scaffolds result in a well-arranged distribution of diverse cell types that can be placed on to the supporting material, simulating tissues with multiple cell types or the interface between two tissues. From this review, it can be concluded that a wide range of phytopharmaceutical/drug-loaded or cell-laden biomaterials can be designed and modified to develop structural integrities with catered geometries for accelerated wound healing as well as prosthetics that will suit patient need and are economically feasible. Furthermore, the research is still on going for maintaining the cell viability, improving the structure resolution, and reducing the cost and fabrication time of newer bioink formulation that can lead to overall improvement in 3D-bioP techniques and finally its clinical utilization. Though the 3D-bioP technique is growing in leaps and bounces, still the clinical success is a huge challenge, and the bridging of gaps is required to make these stimulated biomaterials to pass clinical trial and finally to clinical application.

Acknowledgements We thank the administration of Amity University Uttar Pradesh for their support and encouragement.

Author Contribution PP: resources, validation, visualization, supervision, and writing—original draft. AP: writing and editing. P: material preparation, data collection. JK: review and editing.

Data Availability Not applicable.

Declarations

Conflict of Interest The authors declare no competing interests.

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