Chapter 11
Polymeric Biomaterials for Bioprinting Applications

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1 Introduction

Tissue engineering, regenerative medicine and resettlement are being revolutionised by 3D printing, this allows for the development of very sophisticated, modular, and flexible prosthetics, orthoses, and scaffolds that are patient-specific. For the purpose of tissue engineering, regenerative medicine, and the rehabilitation of patients with severe neurological illnesses, three-dimensional printing (3DP) is becoming a burgeoning technology (such as amyotrophic lateral sclerosis (ALS), craniocerebral trauma, and traumatic spinal cord injury). This is because 3D printing can offer very complex structural designs, tolerant-specific designs, and quick on-demand manufacture at a cheap cost [1–3]. Bioink, the key component of 3D bioprinting, is essential for creating functioning organs or tissue structures. There are several crucial characteristics that must be considered while choosing the bioinks for 3D printing. To create more efficient bioinks for the 3D printing (3DP) of biological or tissue structures, a variety of techniques and property improvements are needed [4]. Materials for 3D printing (3DP) are selected in accordance with the intended use. Materials suitable for 3DP processes include composites, metals, ceramics, and polymers. As polymers

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are typically inexpensive and readily available, they are one of the most regularly used techniques for 3D manifestation, prototyping, validation, and practical applications. One example is the polycaprolactone (PCL) based printing of tissue scaffolds [5, 6]. In this regard, choosing the right biopolymer to use with bioprinting technology is a major difficulty because doing so leaves cytotoxicity and compatibility problems unstudied. In this chapter, we examine novel biopolymers like chitosan, collagen, hyaluronic acid, and silk fibroin. When choosing a biopolymer, it is vital to resemble the biopolymer closer toward nature. For usage in tissue engineering and reincarnate medicament applications, several various Bioink formulations, for example, cell-biomaterials-based Bioinks and cell-based Bioinks, such as cell aggregates and tissue spheroids, have been characterised. The utilisation of ramification polymeric biomaterials, their alterations, and the incorporation of cells and hydrogels have led to the creation of more adaptive bioinks that are printable, mechanically stable after printing, and compatible with live cells [7]. Additionally, biomaterial inks must be readily fabricated, processed, reasonably priced, and commercially available. Keep in mind that the printing process and the intended use of such materials will determine how they must adhere to each of these standards. Thus, a balance would need to be established between all of these variables in order to develop acceptable printed biomaterials with robust properties for a variety of biomedical application demands [8, 9]. The complex geometrical data received from medical imaging techniques like as X-radiation image, nuclear magnetic resonance imaging (NMRI), and micro-computerised tomography scan (μCT scan) may be used to create and evolve such complex 3D tissue architectures in computer-aided design. The ability to create personalised designs for specific patients, high levels of precision, low costs, and the quick manufacturing of complex structures on-demand are all well-being of 3D bioprinting in biomedical engineering [9, 10]. Due to their capacity to mimic the cellular environment and the wide range of processing choices available for polymeric systems, polymers have made up the great majority of the materials utilised in bioprinting under ambient or only moderately harsh chemical and environmental conditions. Additionally, included are applications for bioprinting, cartilaginous bioplants, wound healing bio-bandages, and tissue engineering solutions. Additionally, it is applied in pharmaceutical manufacturing, cancer research, and tissue transplantation via bioprinting. The natural polymers used as inks in bioprinting are covered in great depth in the current study. The present study evaluates the benefits and drawbacks of recently developed polymeric biomaterials with 3D bioprinting technology [11]. Figure 1 shows the year-wise milestones of 3D printing for tissue engineering.

1.1 Biomaterials and Its Classification

A biomaterial is any material, natural or artificial, that is all or part of a living thing or a biomedical device and that carries out, enhances, or replaces a biological function. The term "biomaterials" refers to a broad variety of tissue engineering techniques that allow the evolution of materials for the repair or replacement of different living system

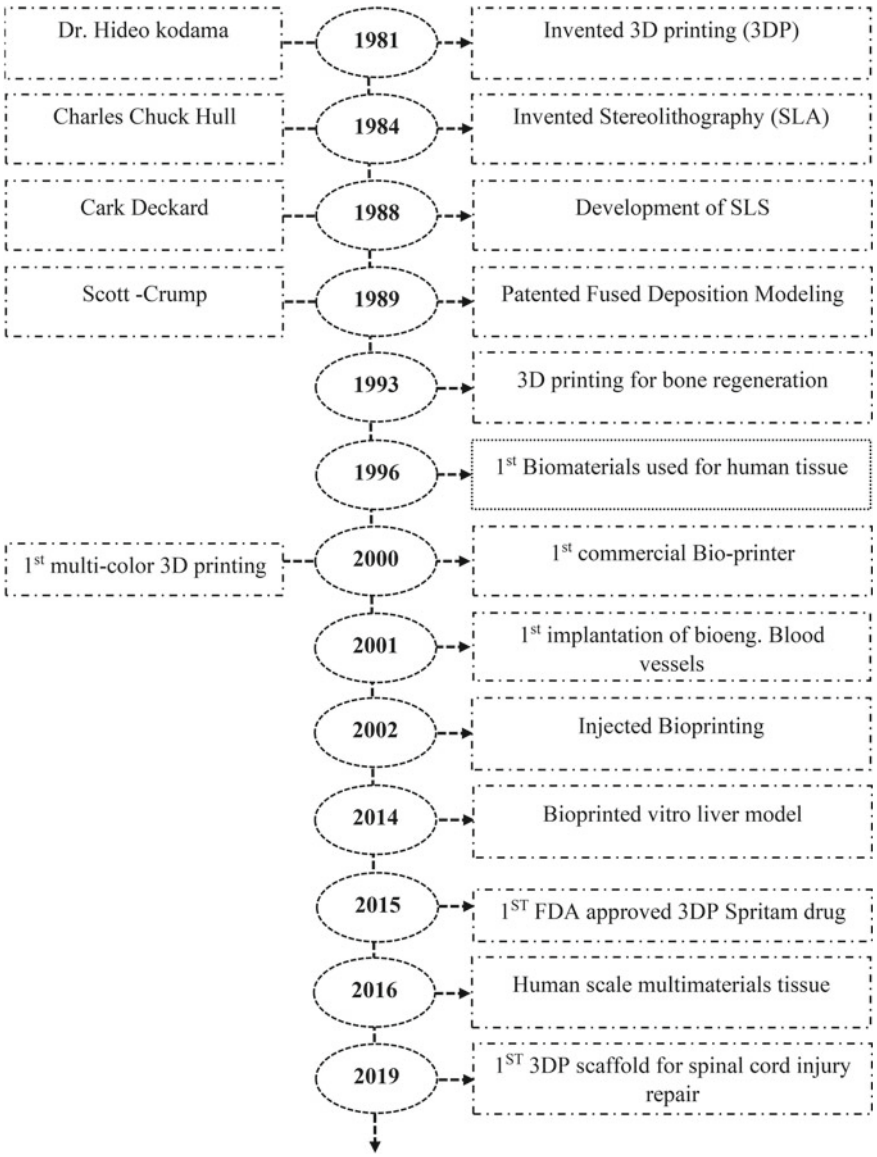

Fig. 1 3D printing milestones for tissue engineering

components. Given that older people are more likely to experience hard tissue failure, demand has increased fast in recent decades as a result of the global phenomenon of population ageing. The main objective is to prolong the life of the implanted biomaterial or to guarantee that it does so until the end of life without faults or the need for revision surgery, contributing to an improvement in the quality of life for the patient.

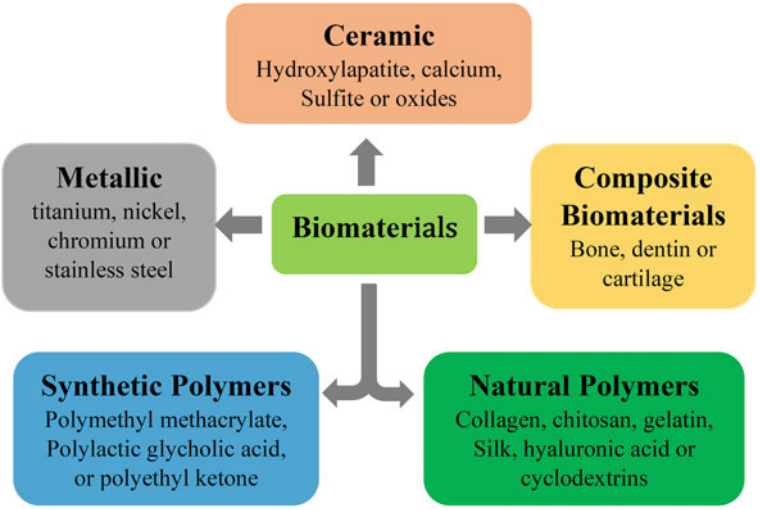

Fig. 2 Types of biomaterials

Therefore, it is necessary for the biomaterial to possess a number of features that are in line with the application in question, including sufficient mechanical reliability, high abrosia and biocompatibility, high wear-tear, low abrasion, and mechanical compatibility [12]. The material classes of polymers, metals, natural materials, composite materials, and ceramics were used to particularly create these biomaterials (Fig. 2). In addition, polymeric biomaterials are segregated into two categories: natural polymer biomaterials and synthetic polymer biomaterials. In cardiovascular devices, polymers are used to replace and stimulate the formation of various soft tissues since they are useful materials for biomedical purposes. Patients have received implants made of several different polymeric materials. They are currently used in a variety of products, including contact and intraocular lenses, artificial hearts, breast prosthesis, vascular grafts, and heart valves [13, 14].

1.2 Polymeric Biomaterials

Polymeric biomaterials have various advantages done other types of materials, including convenience of fabrication, ease clarity of secondary processing, utility with specific mechanical and physical properties, and reasonable pricing. Synthetic and natural polymers are the two kinds employed in biomedical applications. Examples include synthetic polymeric systems including acrylics, polyamides, polyesters, polyethylene, polysiloxanes, and polyurethane. Although synthetic polymers are easily processed, their main drawback is that they are often not biocompatible, which

means that using them is frequently accompanied by inflammatory responses. Utilising natural polymers will help solve this issue. For instance, the biomedical industry uses natural polymers like chitosan, carrageenan, and alginate for tissue regeneration and drug delivery systems [15]. The main types of biomaterials used in tissue engineering include artificial polymers, which include hydrophobic compounds like hydroxy acids, poly-lactic-co-glycolic acid (PLGA), and polyanhydrides as well as naturally occurring polymers including complex sugars (hyaluronan, chitosan), inorganics and polysaccharides. There are further structural or functional subgroups, such as those determined by whether they are hydrogels, injectable, surface-modified, capable of delivering pharmaceuticals, are meant for a particular purpose, etc. [16]. The vast range of potential applications for natural polymers based on proteins or polysaccharides in biomedicine has recently received growing interest. These materials are ideal for a number of purposes in industries including tissue engineering, drug delivery, and wound healing due to their chemical substantiality, structural plasticity, biocompatibility, and excellent availability. Biomaterials that have been taken from plants or animals have also undergone modifications to enhance their structural characteristics or promote interactions with adjacent cells and tissues in order to enhance in vivo performance. This has led to creative uses for surface coatings, controlled medication release, and implanted devices [17]. A growing number of polymeric biomaterials are being produced synthetically and naturally, with a variety of uses including tissue engineering, regeneration, and specialised sectors including medication delivery, nanotechnology, and gene therapy [18]. Because of their unique properties, silks are being used in pre-clinical trials and proof-of-concept experiments in sectors including gene therapy and wound healing, in addition to their traditional usage in textiles. By layer-by-layer or spin-coating techniques, silk films are easily made from fibroin stock solutions and have been used as scaffolds in a variety of tissue engineering applications. When osteoblasts were seeded onto scaffolds using functionalised silk films created by chemically combining Arginyl-glycylaspartic acid (RGD) domains, bone formation was stimulated [19]. Polymer-based biomaterials have taken the place of other materials including metals, alloys, and ceramics due to their low cost, chemical stability, simplicity of processing and reprocessing, and improved corrosion resistance. The medical, biotechnology, food, and cosmetic sectors have all made substantial use of polymer-based biomaterials. Polymeric biomaterials are utilised in medicine for a variety of purposes, including vascular bypass, implantation, wound curing, stitching, catheters, meshes, stents, ligament and tendon renovation, and cardiac procedure valves. Polymeric materials are often categorised as synthetic, natural, or a mix of the two types of polymers for use in biomedical applications. Natural polymers may be derived from both plant and animal sources, with proteins, cellulose, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), cellulose, and silk being the most popular types [20].

1.2.1 Slik Fibroin

Bombyx mori, a silkworm, is the source of the protein fibre known as silk. Spinned by insects and spiders, it is a durable, all-natural biomaterial. Silk fibroin, a protein polymer utilised and investigated for biomaterial applications, is generated from Bombyx mori cocoons. Silk fibroin demonstrates biocompatibility, degrades predictably over time, from hours to years, and is chemically modifiable to modify surface properties or immobilise growth inputs. When moulded into different materials, silk fibroin also possesses exceptional mechanical qualities. Silk biomaterials for various applications can be produced by a number of techniques including the processing of aqueous or organic solvents. Using the techniques outlined in this procedure, silk from Bombyx mori cocoons may be extracted and used to create hydrogels, tubes, sponges, composite materials, fibres, microspheres, and thin films. These materials can be utilised for implantable biomaterials, drug delivery, tissue engineering scaffolds, and in vitro disease modelling [21]. Bombyx mori (silkworm) silk is a special substance that has long been prized for its tenacity and brightness. From ancient times, clinicians have used silk as a suture material. Recently, silk as a biomaterial paid more attention due to a variety of compelling features [22, 23]. These qualities specifically include its biocompatibility, ease of chemical modification, slow rate of in vivo degradation, and capacity for processing into a variety of material forms from either an organic solvent or an aqueous solution [24]. Silk worm silk is more durable than often used polymeric biomaterials like collagen and poly (lactic acid) (PLA). Silk fibres from B. mori have a maximum tensile strength of 740 MPa. Compared to PLA's 28–50 MPa, collagen's 0.9–7.4 MPa ultimate tensile strength is significantly lower. As a result, silk fibroin is an excellent choice of polymer for biological applications. Silk fibres are more resilient compared to the sturdiest synthetic materials [25, 26] (Fig. 3).

(a) Bombyx mori silk cocoon (b) Silk Fiber (c) Silk solution

Fig. 3 Silk fibroin extraction procedure

1.2.2 Collagen

Almost all biological systems include a significant amount of collagen, a crucial protein for all living things. It is a protein that gives bodily tissues rigidity and strength while promoting the development of networks along cellular structures. Because of their great biocompatibility, exceptional biodegradability, and minimal immunogenicity, collagen molecules were shown to be the best candidates for real therapeutic alternatives in contemporary biomedical and biotechnological applications. These factors make collagen one of the biopolymers that are now being researched the most in the medical industry [27]. In both humans and animals, collagen makes up the majority of the structural proteins. It is an important part of the ECM and makes up around 30% of all mammalian proteins. It supports the structural integrity of both hard and soft tissues, such as blood vessels, cartilage, tendon, bone, and ligaments, thanks to its distinctive fibrillar structure [28]. Collagen is only marginally immunogenic, with fibrillose collagen being less immunogenic than smaller molecules as a result of the encapsulation of possible antigenic sites during its auto-polymerisation. In biomedical applications, cross-linking improves a material's biocompatibility, degradability, and resistance to collagenase activity [29]. In order to strengthen its mechanical strength and resistance to enzymatic degradation, extracted collagen must be cross-linked in contrast to decellularised collagen, which has been naturally polymerised in vivo. Cross-linking can be done in a number of well-known ways, including physically using UV or heat treatment, chemically using formaldehyde and glutaraldehyde, or biologically using cross-linking enzymes like transglutaminase [30]. In order to create fibres that mirror the biological properties of ECM at submicron to nanoscale sizes, collagen kinds I, II and III were electrospinning. The collagen fibres were developed with a 67-nm binding pattern that is identical to genuine collagen in order to produce collagen type electrospun biomimetic scaffolds with melodious porosity, mechanical strength, and fibre alignment to topographically direct tissue growth. Electrospun collagen scaffolds have also demonstrated the ability to sustain cellular development [31, 32]. A hydrogel is a 3D polymer network with a large fluid-holding capacity. Due to the hydrophilic functional groups on their polymeric backbone, polymeric hydrogels' major therapeutically significant properties are their capacity to hold water, resistance to disintegration via cross-linking, and flexibility equivalent to that of natural ECM. Because collagen hydrogels have a structural similarity to tissue, they are being researched as biomimicry 3D scaffolds to aid in cell progress [33, 34]. The preservation of cells and bioactive substances by collagen hydrogels makes them desirable scaffolds for tissue engineering. They are essential for the growth of bone and cartilage, functioning as carriers for chondrocytes from cows to offer, among other things, structural integrity and pain-free cartilage articulation [35]. Due in large part to their great biocompatibility, minimal immunogenicity, and diversity in terms of structure, collagen-based materials are now at the forefront of biomaterials used in tissue engineering and regenerative medicament. Applications for collagen in areas including tissue regeneration, medication administration, and wound healing are becoming more and more varied because of improvements in extraction and scaffold composition. In order to increase the

in vivo effectiveness of collagen-based scaffolds, future research is anticipated to concentrate on enhancing the mechanical strength, drug transport capacities, and biodegradability of these materials.

1.2.3 Gelatin

Biopolymer made of gelatin is entirely absorbed, biodegradable, and biocompatible. Gelatin is suited for a variety of tissue engineering applications because of these qualities, which have generated a lot of attention. Gelatin is a crucial functional biopolymer that is frequently added to meals to increase their elasticity, consistency, and stability. Together with the skin and bones of land animals, it may also be obtained from fish and insects. To make type A and type B gelatins, respectively, the acid and alkaline procedures are often utilised in gelatin production [36]. The process of thermally denaturing collagen, the most prevalent and essential protein in the animal kingdom, yields gelatin, a dietary item that is essentially entirely composed of protein [37]. High molecular weight gelatin is a necessary hydrocolloid with a polypeptide chain. Because of its capacity to thicken and gel, it has grown in favour among the general public and is used in a range of culinary products. In contrast to other hydrocolloids, which are typically polysaccharides, gelatin is a digestible protein that contains all of the necessary amino acids minus tryptophan. Because of exposure to many environmental conditions, most notably temperature, the amount of amino acids can differ between species, especially for proline and hydroxyproline [38]. From a variety of collagen sources, gelatin may be produced. The main commercial sources are fish, cow bones, pig skins, and pig hides. It might thus originate from both agricultural and non-agricultural sources. Seaweed extracts and other substances referred to as "vegetable gelatin" have no biological connection to gelatin, and gelatin has no plant origins [39]. The name "gelatin" refers to a group of dietary protein products made by hydrolysing collagen from cold-blooded animals like fish and insects, which is found in their bones and skins. There are two methods for extracting the fish and insect gelatin: an acid procedure and an alkaline technique. When discussing the extraction of fish gelatin, the term "acid process" refers to both the extraction carried out in an acid medium and, in certain situations, the use of an acid pre-treatment before the extraction. The extraction can be done in an alkaline, neutral, or acid medium thanks to the alkaline procedure, which involves pre-treating fish skin with an alkaline solution and often neutralising it with an acid solution [40, 41]. The chemical attributes of gelatin are connected to its functional qualities. The renaturation of gelatin subunits during gelling depends on proline and hydroxyproline. The distribution of Gelatin's molecular weight and amino acid concentration affects all of its properties, including gel strength, viscosity, setting behaviour, and melting point. Gelatin containing a lot of amino acids tends to have a greater melting point and gel strength as a result [42]. The majority of gelatin purchased by the pharmaceutical sector is often used for the production of tablets, coatings for tablets, granulation, and hard and soft gelatin capsules (Softgels) where it improves the preparation's flavour and prevents oxidation. The research examining the use of gelatin-based

biomaterials for the engineering of the heart, liver, skin, and cornea is covered in this topic. Gelatin and injectable fillers are discussed as possible treatments for wounds. Due to their aptitude for self-renewal and propensity to specialise into specific cell types, stem cells offer considerable promise for use in tissue regeneration. In order to increase the production of therapeutic proteins or to initiate gene silencing, respectively, gelatin-based delivery methods have been successful in delivering genes and siRNA. Overall, gelatin-based drug delivery methods have shown to be quite useful and adaptable in a variety of biological applications [43].

1.2.4 Chitosan

Arthropod exoskeletons are the primary source of chitin, although it may also be found in fungus, insects, and mushrooms. The exoskeleton of crab shells is made of chitosan, a glycosaminoglycan-like substance that is an N-deacetylated derivative of chitin and a member of the polysaccharide family. Due to its functionality, biocompatibility, biodegradability, and antibacterial qualities, chitosan has received extensive research as a natural biomaterial for several biomedical applications [44]. Biomaterials, or those made from biological sources, play a specific role in the search for the perfect dental materials because of their wide range of applications and innate biocompatibility. Chitosan is a relatively new substance that is mostly obtained from the exoskeletons of life, notably anthropods and fungus, etc. We are now able to use more biomaterials that are simple to adapt for human use, have fewer side effects, and have greater therapeutic results because of evolving technology and knowledge. Chitosan has more and more uses in medicine, thus research into its uses in dentistry also has to be pursued with greater zeal. Chitosan may be a genuine blessing in disguise for dentistry and find application in therapies ranging from preventative to regenerative dentistry in many of its specialisations, thanks to its features of being antibacterial, biocompatible, biodegradable, osteoconducting, etc. [45]. The most valuable by-product of chitin is chitosan, a natural polysaccharide. It is an effective microbiological agent with improved and increased qualities. After roughly 50% deacetylation, chitin undergoes 70% alkaline deacetylation to produce chitosan as shown in Fig. 4. The linear structure of chitosan is made up of a copolymer of glucosamine and N-acetylglucosamine. In the pharmacological, biomedical, cosmetics, hair care, acne treatment, and agricultural sectors, chitosan has a wide range of uses. In the pharmaceutical industry, it is used to accelerate the development of fruits and vegetables [46].

1.2.5 Hyaluronic Acid (HA)

Hyaluronic acid (HA) has been used more frequently recently as a biomaterial in both therapeutic and scientific purposes. Due to its great abundance in several tissues and ease of chemical manipulation, HA has become a prominent material platform for a

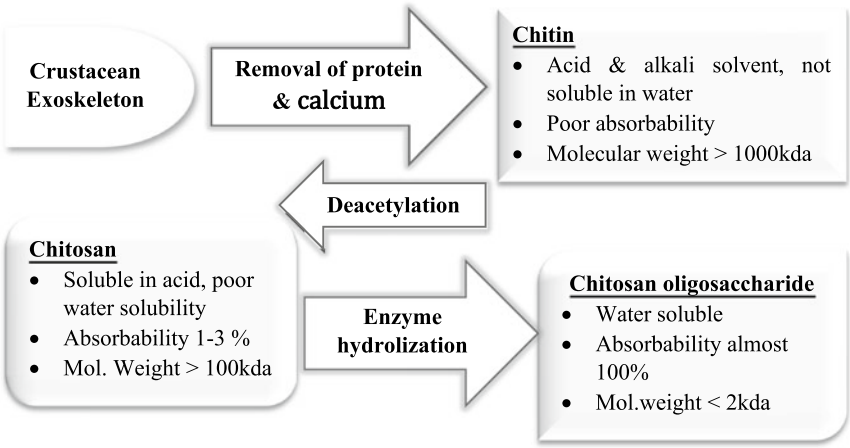

Fig. 4 Schematic diagram of formation of chitosan

variety of activities, including regenerative medicine, medication delivery, and scaffolding for cell culture. With its unique physicochemical characteristics and capacity to organise other matrix proteins, hyaluronic acid (HA) has long been recognised for its potential to influence tissue dynamics and remodelling. By activating cellular HA receptors, which may transmit signals that change cell survival, spreading, adhesion, and migration, HA can instead have a far more direct and focussed impact on how cells behave. Cells then alter HA by controlling its synthesis and breakdown using a specialised suite of enzymes. Taking into account all of these different mechanisms of control is necessary for the best design of HA-based biomaterials [47] (Fig. 5).

Nearly all physiological tissues and fluids contain hyaluronic acid (HA), a linear polysaccharide whose molecular weight (MW) varies depending on the type of tissue. Hyaluronic acid is sometimes referred to as hyaluronan. A sign of HA's biological significance and potential for therapeutic use is the fact that it is expressed basically everywhere. It is suitable for usage in a range of applications requiring conjugation or cross-linking because of its three orthogonal functional moieties (hydroxyl, carboxyl, and amide). These permit a variety of chemical changes [48]. Glucuronic acid and N-Acetyl-D-glucosamine are joined by alternating 1–3 and 1–4 glycosidic

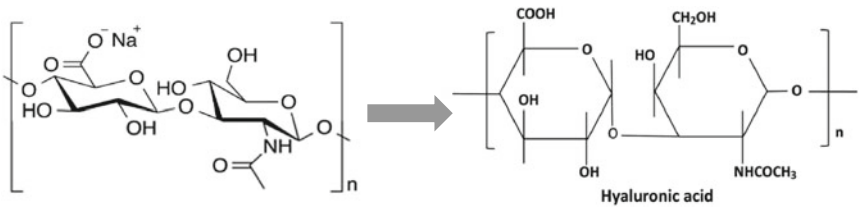

Fig. 5 Structure of hyaluronic acid

linkages to form the repeating disaccharide unit that makes up hyaluronic acid, a linear polysaccharide. The extracellular matrix of connective and epithelial tissues contains a glycosaminoglycan, which is its source. The material that grounds dermal cells and fibres into the skin is made up in part of hyaluronic acid, which is also present in the epidermis. In actuality, while being present throughout the body, hyaluronic acid is mostly produced by fibroblast cells in the skin, where it is primarily found [49]. Hyaluronic acid also has the ability to reduce inflammation and speed up the healing of wounds. The lower layers of the epidermis contain hyaluronic acid, which is thought to have a regulatory and modulatory function in the body due to its capacity to bind to proteins and cell surface receptors. The dermis's large hydrophilic polysaccharide, hyaluronic acid was once thought to contribute to the mechanical properties of skin by absorbing water. A variety of significant biological roles that this molecule plays in maintaining skin homeostasis are now being revealed by researchers [50]. The quality of the implanted cartilaginous extracellular matrix is improved by a bioink composition based on hyaluronic acid that permits 3D bioprinting. The ability to create 3D cell-hydrogel structures in 3D bioprinting that are strong enough requires the use of bioinks with high concentrations of polymeric components [51].

1.3 Tissue Engineering

Tissue engineering, a branch of biomaterial engineering, makes use of tissue synthesis to repair or replace whole tissues, such as skin, muscle, cartilage, bone, blood vessels, and many more. Tissue engineering, which is frequently used in conjunction with the term "regenerative medicine," is generally defined as the process of designing or directing the repair of tissues. However, the term can also refer to technologies used outside of the body, such as the construction of tissue constructs for in vitro research. The overlap results from the necessity of biomaterial technologies for the engineering of living tissues. Tissue engineering is an interdisciplinary field that aims to create biological tissues that improve, maintain, or repair tissue function or the function of a whole organ [52]. The following example illustrates how the basic concept of tissue engineering is represented in Fig. 6. A cell culture system or a bioreactor must be used to grow the isolated cells once they have been obtained from a source (allogenic, xenogenic, or autologous) (expansion in vitro). The enlarged cells must next be seeded into a carrier or matrix, which provides structural support and allows for the inclusion of the proper media (rich with nutrients and growth factors). The cells generate new tissues by differentiating, multiplying, and migrating to the carrier, which replaces the old tissues. The resulting tissue engineering construct is then used to create replacement tissue that is subsequently transplanted back into the patient [53].

Another way that nanotechnology is used in medicine is through tissue engineering (nanomedicine). By employing appropriate scaffolds comprised of nanomaterials and growth hormones, damaged tissues may be replicated or replaced. To build new tissues or organs to replace existing ones is the emphasis of the field of

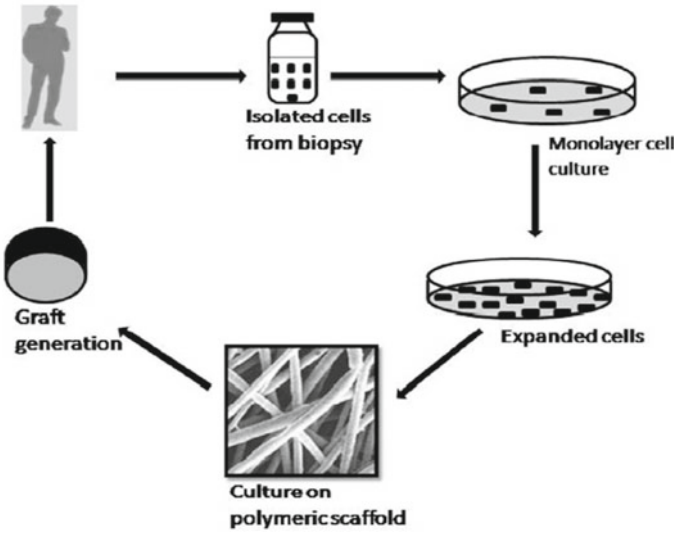

Fig. 6 Basic principle of tissue engineering

tissue engineering in nanotechnology. This initiative may take the place of currently used conventional therapeutic modalities like organ transplants or artificial implants. The host immune system can be suppressed, host tolerance can be induced, or immunomodulation of the tissue-engineered construct can assist avoid immunological responses in connection to these procedures [54]. Complex 3D working live tissues may now be printed using biocompatible materials, cells, and auxiliary components. With 3D Bioprinting, regenerative medicine is able to overcome the shortage of transplantable tissues and organs. When compared to non-biological printing, 3D bioprinting is more difficult due to considerations including material choice, cell types, growth and differentiation factors, and technological worries about the sensitivity of living cells and the formation of tissues. Combining engineering, biomaterials science, cell biology, physics, and medical technologies is important to find solutions to these issues. A range of tissues, including multilayered skin, bone, vascular grafts, tracheal splints, heart tissue, and cartilaginous structures, have been created and transplanted before using 3D bioprinting [55]. Tissue engineering is quickly growing into a sector with enormous potential, addressing particular medical requirements like organ failure or significant tissue damage. The objective of tissue engineering is to produce functional tissues that allow for the repair, upkeep, or enhancement of damaged organs or tissues. Diseases and disorders that might usually render a patient helpless or kill them can now be treated, thanks to tissue engineering. If spontaneous regeneration is impossible due to evolution, it allows tissue regeneration. Tissue engineering enables the body to repair itself, in essence. Tissue engineering is most frequently used to produce tissues that may be used to replace or repair bodily tissues that have lost some or all of their functionality. There are still

many applications for tissue engineering, such as the creation of extracorporeal life support systems (such as bioartificial liver and kidneys), in vitro disease models, tissues for drug screening, smart diagnostics, and individualised medicine [56].

2 3D Printing

Numerous terms and descriptions that are similar to 3D printing are used to describe it, including additive manufacturing and fast prototyping. However, they all characterise additive manufacturing as the key concept that sets it apart from traditional subtractive techniques. The technology of 3D printing (3DP) allows for the creation of 3D solid things from digital files in any shape or geometry. The computer is given a computer-aided design (CAD) model of the design that will be produced. The term that broadly refers to printing on a range of materials, including polymers, plastics, ceramics, metals, and composites. In 1984, Chuck Hull invented the stereolithography technique, which involves curing photopolymers using UV lasers to build layers [57]. A 3D printer is made up of a variety of parts that work together to create the desired result from an input digital file. This is a list of a 3D printer's basic components: Extruder, hot end, print bed (tray), and filament. A computer file is converted into a real thing through the lengthy and intricate process of 3D printing. The procedure is broken down into the four steps listed below: Step-1: Modelling: CAD files may be produced either from scratch using a 3D modelling application or by starting with a 3D model produced by a 3D scanner. In any case, the application generates a file that is delivered to the 3D printer. Software separates the design into hundreds of horizontal layers, maybe thousands, as the process progresses. Create 3D models with 3D modelling software (such as CAD software), and download the model files (STL files). Step-2: Slicing: To slice the model, use a programme like ideaMaker. IdeaMaker creates a GCode file. Step-3: Printing: In order to prepare the printer for printing, upload the slice file and calibrate the printer. Step-4: Post-processing: Post-processing is the final stage in 3D printing. The post-processing for fused filament fabrication (FFF) 3D printing includes the following procedures, albeit not all of them must be carried out: removing the support, sanding, colouring, polishing, welding, and assembling everything. Some methods for forming the layers include melting or softening the substance, while others employ cutting-edge technology to cure liquid materials. Each approach has benefits and downsides of its own. Some frequently used technologies include the ones listed below: Selective laser sintering (SLS), multi-jet modelling (MJM), fused deposition modelling (FDM), stereolithography (SLA) [58]. For the manufacturing of polymer components, including prototypes and functional constructions with complex geometries, 3D printing methods are frequently employed. Due to their low cost, light weight, and processing flexibility, liquid or low melting point polymer materials for 3D printing are often employed in the sector. Being inert materials, polymer-based materials have traditionally played a significant role in biomaterials and medical device items. They helped the devices work effectively and provided mechanical support for numerous

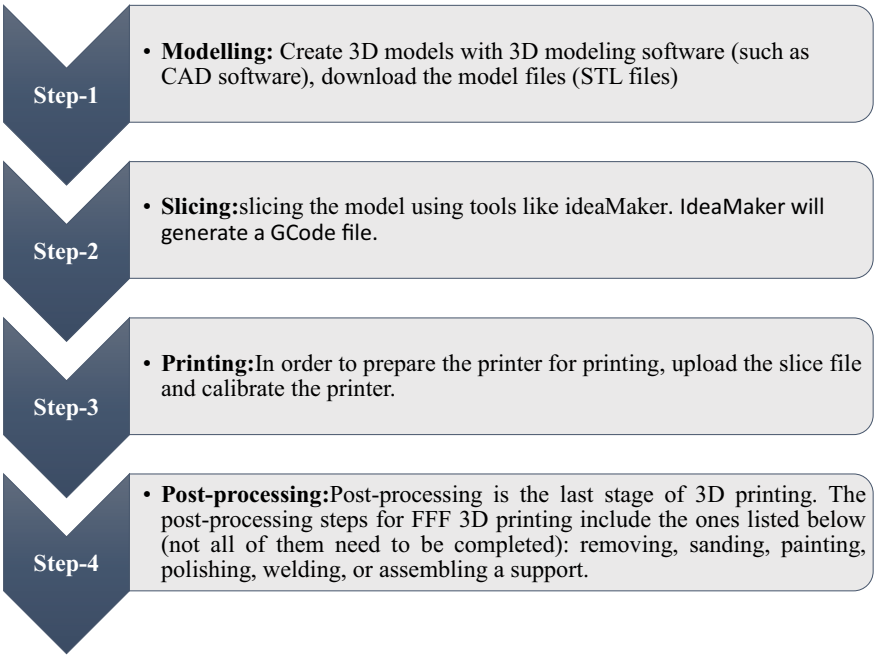

Fig. 7 Layout of a 3D printing model

orthopaedic implants [59, 60]. The natural structure of the skin can be more economically replicated by using 3D printing technology. On 3D printed skin, chemicals, pharmaceuticals, and cosmetics may be examined. Animal testing of products is thus unnecessary. The researcher will therefore be able to get accurate data by using a copy of the skin [61]. By using 3D printing technology, bony holes in the cartilage or bone brought on by illness or damage may be replaced. In contrast to employing auto-grafts and allografts, the goal of this treatment is to create bone, preserve bone, or improve bone function in vivo [62] (Fig. 7).

2.1 3D Bioprinting

3D Bioprinting (3DP) is one of the more advanced technologies which is out there where people are trying to fabricate scaffolds using this. The process of 3D bioprinting is slightly different from 3D printing. In 3D bioprinting, you will thus use cells together with the ink, whereas in 3D printing, you would simply print with standard ink and then seed cells on it. Extrusion-based, droplet-based, and laser-based 3D bioprinting techniques are grouped together as such. Bioinks that are filled with cells are patterned using laser radiation. The laser induced forward transfer, often known as the LIFT, is the method employed the most frequently in laser-based

bioprinting. It is more common to use droplet-based bioprinting, which is also where 3D printing really began. This may be divided into two categories: drop-on-demand inkjet printing and continuous inkjet printing. Inkjet bioprinting offers the benefit of having incredibly high resolution and rapid printing. Moreover, it is relatively inexpensive. By using drop-on-demand procedures, it may introduce gradients in cell concentration. The most popular type of bioprinting is extrusion-based, and in this case, either mechanical or pneumatic pressure is utilised to extrude the Bioink from a nozzle. The mechanical force required to push the ink out might be a screw or a piston. Due to its capacity to control the hierarchical assembly of 3-dimensional biological structures for tissue building, 3D bioprinting is a method that has attracted significant interest for tissue engineering applications. In 3D bioprinting, functional components are positioned spatially and biological materials, biochemical, and live cells are carefully positioned layer by layer to create 3D structures. Three-dimensional bioprinting uses a variety of techniques, such as biomimicry, autonomous self-assembly, and microscopic tissue building blocks. Researchers are working on these approaches to build 3D functioning, living human structures that are suitable for therapeutic tissue and organ function restoration. The printing of fragile, living biological materials requires a major adjustment of printing technology designed for molten polymers and metals. Since extracellular matrix (ECM) components and many cell types have unique micro-architectures, it is difficult to mimic biological functions [55] (Fig. 8).

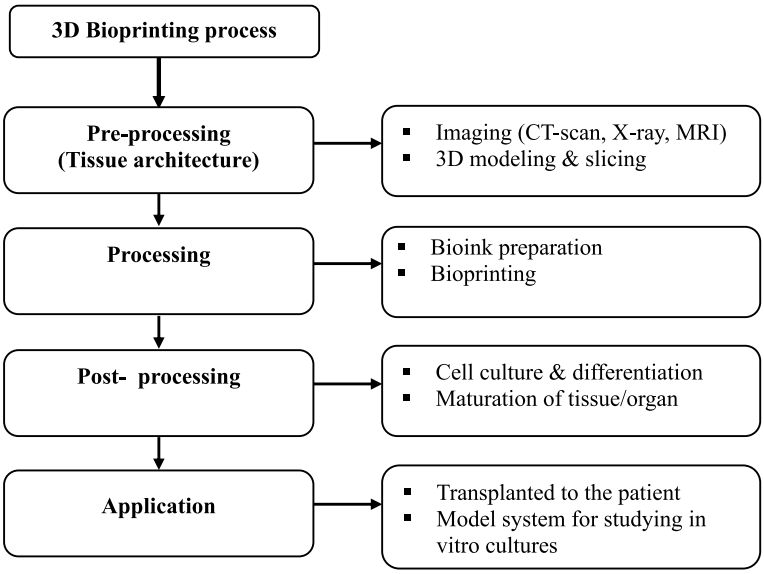

Fig. 8 Process of 3D bioprinting

2.2 Bioink Requirements for 3D Bioprinting

Bioink is a crucial component in the 3D bioprinting process, as it serves as a scaffold or support material for the living cells that will eventually develop into functional tissues and organs. The requirements of bioink for 3D bioprinting depend on several factors, including the type of tissue or organ being printed, the type of printer used, and the specific properties of the bioink itself. However, some general requirements of bioink for 3D bioprinting include:

1. *Biocompatibility*: Bioink must be biocompatible with the living cells being printed to prevent cytotoxicity or cell death. The bioink should also be non-immunogenic to prevent an immune response.
2. *Printability*: Bioink must be able to be printed with high precision and accuracy to create the desired 3D structure. The viscosity, surface tension, and other physical properties of the bioink can affect its printability.
3. *Degradability*: Bioink must be able to degrade over time to allow the cells to grow and mature into functional tissues. To maintain optimal tissue growth, the degradation rate should coincide with the rate of tissue synthesis.
4. *Mechanical properties*: Bioink has to have the right mechanical qualities to give the cells structural support as they develop and differentiate. The mechanical properties should mimic the native tissue being printed, such as stiffness or elasticity.
5. *Sterilisation*: Bioink must be sterilised to prevent contamination or infection during the printing process. Sterilisation methods may vary depending on the type of bioink used.
6. *Versatility*: Bioink must be versatile enough to accommodate a variety of cell types and tissue structures. Some bioinks may be tailored to specific tissue types or cell types to enhance tissue development.
7. *Functionality*: Bioink should have suitable chemical and biological cues that can promote cell adhesion, proliferation, differentiation, and ECM production. It should also be able to support vascularisation and innervation in engineered tissues and organs.

Overall, bioink must meet strict requirements to enable successful 3D bioprinting, and ongoing research is focussed on developing bioinks that can more closely mimic native tissue structures and properties. In general, the needs of bioink for 3D bioprinting are complicated and varied, necessitating careful selection and optimisation of biomaterials and processing conditions for particular applications [63].

2.3 Application of Natural Polymeric Biomaterials in Bioprinting

Natural polymeric biomaterials are widely used in bioprinting applications due to their biocompatibility, biodegradability, and ability to mimic the extracellular matrix of living tissues. Here are some of the applications of natural polymeric biomaterials in bioprinting:

1. *Tissue engineering*: Natural polymeric biomaterials are also used in bioprinting applications to regenerate damaged or diseased tissue. Natural polymeric biomaterials such as collagen, gelatin, alginate, and fibrin are commonly used to create scaffolds for tissue engineering applications. These scaffolds can be designed to match the properties of specific tissues, promoting the growth, proliferation, and differentiation of cells. For example, fibrin-based hydrogels have been used to create vascular networks, while alginate-based hydrogels have been used to regenerate liver tissue [64].
2. *Scaffold fabrication*: Natural polymeric biomaterials such as collagen, gelatin, alginate, and fibrin are commonly used to create scaffolds for tissue engineering. These scaffolds provide a 3D structure that can support cell growth, differentiation, and tissue regeneration. For example, collagen-based scaffolds have been used to fabricate cartilage tissue, while alginate-based scaffolds have been used to create pancreatic tissue [65].
3. *Drug delivery*: Natural polymeric biomaterials can be used as drug delivery vehicles in bioprinting applications. These biomaterials can be modified to encapsulate and release therapeutic molecules in a controlled manner, improving drug efficacy and reducing side effects. For instance, growth factors have been delivered to cartilage tissue using hydrogels made of gelatin [66].
4. *Vascularisation*: Natural polymeric biomaterials such as gelatin and fibrin can be used to create vascular networks within bioprinted tissues. These networks can improve nutrient and oxygen delivery to cells, leading to better tissue viability and function [67].
5. *Organ printing*: Natural polymeric biomaterials can be used to create functional tissues and organs through bioprinting. These biomaterials can provide an environment that is conducive to cell growth and differentiation, resulting in the development of intricate 3D structures [55].
6. *Organ-on-a-chip*: Organ-on-a-chip devices are also being developed using natural polymeric biomaterials. These systems can be utilised for disease modelling and medication screening because they replicate the structure and operation of live organisms. For example, collagen-based hydrogels have been used to create a liver-on-a-chip system [66].
7. *Wound healing*: Natural polymeric biomaterials such as chitosan and alginate have been used in bioprinting for wound healing applications. These biomaterials can be used to create scaffolds that promote cell migration, proliferation, and differentiation, leading to improved wound healing outcomes [68].

2.4 Future Scope and Summary

Natural polymeric biomaterials are materials that are derived from natural sources and are used in medical applications. These materials have gained considerable interest in recent years due to their biocompatibility, biodegradability, and low toxicity. They are employed in a number of medical procedures, including implanted medical devices, medication delivery, tissue engineering, and wound healing. One of the major advantages of natural polymeric biomaterials is that they can be easily modified to meet specific requirements. For example, the mechanical properties of these materials can be tuned by adjusting their composition or processing conditions. Natural polymeric biomaterials have demonstrated tremendous promise as building blocks for bioprinting because of their biocompatibility, biodegradability, and capacity to replicate the extracellular matrix (ECM) of real tissues. Aiming to improve patient outcomes in regenerative medicine, bioprinting is a fast-developing technology that shows promise for the creation of functioning tissues and organs. Collagen, gelatin, alginate, chitosan, and hyaluronic acid are some of the most popular natural polymers for bioprinting. These materials may be coupled with other materials, including synthetic polymers, cells, and growth factors, to improve their qualities. They have distinctive features that make them useful for a variety of applications. One of the key challenges in bioprinting with natural polymeric biomaterials is achieving adequate mechanical strength and stability in the printed constructs. To enhance the mechanical qualities of the printed constructions, researchers are creating novel cross-linking and curing techniques. The creation of novel bioinks that can be utilised to produce complex structures using various cell types and biomaterials is another area of ongoing study. Advances in bioprinting technology, such as the use of multi-material printing and advanced imaging techniques, are also expanding the possibilities for creating functional tissues and organs. Overall, the future of natural polymeric biomaterials for bioprinting looks promising, with continued advances in material science, bioprinting technology, and tissue engineering expected to lead to the development of new and innovative biomaterials and its applications.

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