Review

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Review

Narrative Review and Perspective: The State of Art and Emerging Opportunities of Bioprinting in Tissue Regeneration and Medical Instrumentation

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Abstract: 3D printing was introduced in the 1980s, though bioprinting started developing a few years later. Today 3D bioprinting is making inroads in medical fields, including production of biomedical supplies intended for internal use, such as biodegradable staples. Medical bioprinting enables versatility and flexibility on demand and is able to modify and individualize production using several established printing methods. A great selection of biomaterials and bioinks is available, natural, synthetic and mixed; they are biocompatible and non-toxic. Many bioinks are biodegradable and they accommodate cells so upon implantation they integrate within the new environment. Bioprinting is suitable for printing of tissues using living or viable components, such collagen scaffolding, cartilage components, cells, and also for printing parts of structures, such as teeth, using artificial, man-made materials that will become embedded *in vivo*. Bioprinting is an integral part of tissue engineering and regenerative medicine. The addition of newly developed smart biomaterials capable of incorporating dynamic changes in shape depending on the nature of stimuli led to adding the 4th dimension of time in the form of changing shape to the three static dimensions. 4D bioprinting is already making significant inroads in tissue engineering and regenerative medicine, including new ways to create dynamic tissues. Its future lies in constructing partial or whole organ generation.

Keywords: tissue engineering; regenerative medicine; 3D printing and bioprinting; biomaterials; bioinks; 4D bioprinting; smart materials; smart stimuli; smart design

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1. Introduction

I am not a bioengineer, I am a pathologist and cell biologist interested and pursuing research in tissue repair. I have been fascinated by 3D printing and its possibilities, and new venues and promises it provides for the future of medicine. I find the idea that organs can be bioprinted and implanted into human beings very appealing – it would mean that more people can live better lives if they can receive bio- and immunocompatible organs printed on demand and would not have to wait for somebody to die so they can get a chance to live (and take many medications to keep the new organ accepted). The availability of bioprinted organs would solve not only the shortage of donor organs (at least to some degree) but would ease ethical questions surrounding organ transplantation. When I started working on this review, I realized pretty quickly that this is not going to be a comprehensive review – simply because it is such a rapidly evolving field and with so much progress and new technology a comprehensive review would amount to a book and not to a journal article. I decided to include the most exciting and new technologies in the bioprinting field.

Bioengineering, a rapidly developing area of engineering, specializes on creating and/or manufacturing biomaterials that can used in medicine, both human and veterinary, for tissue repair and reconstruction of structures impaired or destroyed by disease or trauma. Both artificial and natural materials are used in bioengineering, though human-made and non-biological materials have to be biocompatible, able to become part of the newly formed tissue or at least be degradable and non-toxic (and nonimmunogenic if possible). Biomedical engineering concentrates on the development and manufacture of medical devices, including those which can replace organs, such as artificial heart, pacemakers, dental and orthopedic prostheses. One can easily envision a situation where it is difficult to distinguish between the two fields. A bioprosthetic Edwards heart valve developed for transcatheter aortic valve replacement is a good example where biological tissue (bovine pericardium) serves as a medium to insert a balloon-expandable cobalt-chromium frame into human heart via catheter. Though the pericardium is not quite bioengineered one can envision a scenario where a heart valve would be replaced by a valve consisting not only of bioengineered pericardium, but other components of the valve as well.

3D printing came on the scene 40 years ago and it has been widely used and applied to numerous purposes in many industries, including biomedical fields (where it is usually called 3D bioprinting, both for production of medical devices and of means for tissue regeneration. The evolution of 3D bioprinting took longer time because of inherent difficulties connected to special needs, such as strict sterility of printing equipment and biomaterials and cells, maintaining viability of cells, biocompatibility and lack of toxicity just to name a few issues. The more recent appearance of so called 4D bioprinting where the fourth dimension is time-associated brings new features to bioprinting enabling changes in shape of bioprinted items depending on stimuli encountered *in vivo*. Organ formation and production for use in transplantation is one of the ultimate goals of bioprinting as discussed below.

2. 3D printing

Three-dimensional (3D) printing, also called additive manufacturing has been around for more than 40 years, since the early 1980s [1,2]. Products are constructed by addition of material in layers. Essential part of this process is using computer-aided (CAD) design software modeling which instructs the 3D printer to create and print the computer-designed object.

Though this process was initially rather slow, it turned out to be suitable for R&D of new products and printing of three-dimensional objects. 3D printing is undergoing a renaissance with several types of production suitable for a great range of manufacturing of variety products by several industries. This technology found application in regenerative medicine, more specifically in orthopedics and dentistry early on [3]. Since then, 3D printing has undergone a lot of progress in terms of methodology and number of applications in many other fields, including biomedical devices besides those used in tissue regeneration. Though 3D bioprinting implies creation of biomaterials and tissues we included a brief section discussing the role of 3D printing in the development and application of medical devices as they are indispensable to bioengineering and as they are (or have the potential to be) used together with bioprinted tissues or be merged with organs and tissues in the body because of their biocompatibility, and similarity to tissues such as bone and cartilage. 3D printing is still developing, for one it is rather slow and thus not suitable for mass manufacturing, but it is quite versatile and many modifications are used in many fields and industry. There are certain features common to most if not all types of 3D printing. 3D printing is easily adaptable in terms of customization, prototyping and complex geometrics. A variety of materials can be adapted to 3D printing, from metals, thermoplastics, ceramics, fibers, polymers to biomaterials and extracellular matrix, the last two with or without cell content. It can be easily customized with prototypes developed in short periods of time, though as pointed above the printing itself might be slow. The fast improvements in software and 3D printing facilities which can be accommodated near customers provide definitive advantages when it comes to production itself and to development in techniques and new prototypes and products themselves [1]. For better understanding of the current applications and future possibilities which may expand the use of bioprinting we provide a brief summary of the most commonly used techniques and technologies available by 3D printing.

2.1. Types of 3D Printing

UV laser used to solidify liquid photopolymer in layers is the basis behind so called stereolithography (SLA). This technique results in solid objects which are highly polished and have great resolution. It is suitable for creation of detailed prototypes and in manufacturing of dental and medical equipment using biocompatible resins [4].

Digital light processing (DLP) uses digital light projecting rather than laser to accomplish the same as SLA but it is much faster, though the resolution is not as good. The speed with additional advantages such as production of thinner layers and enlarged number of suitable materials make DLP suitable for jewelry detailing and for dental applications [5].

Fused deposition modeling (FDM) also known as melt extrusion where melted multiple layers of hot thermoplastic filaments are extruded through a hot nozzle. Many filamentous materials used widely in bioprinting are processed this way, e.g., polylactic acid, variety of polymer composites, and ceramic materials, just to name a few examples. Among the main advantages are flexibility, speed of manufacturing. It is biggest applications are in the aerospace and healthcare industries [6].

What makes laser ablation an ablation is selective removal (i.e., ablation) of layer of material from a larger block and shaping into desired form with a high-power laser beam. This technique turns out to be most useful for the aerospace industry [7].

High-intensity laser beam is used to polymerize liquid resins (that's the designation of this method as multiphoton polymerization) allowing precise control during several biomedical processes, e.g., tissue engineering, and activities (micro-optics and microfabrication just to name a couple of them) [8].

2.2. 3D Bioprinting in Biomedical Applications

I do not have to educate readers of this journal about bioengineering and perhaps not even about 3D bioprinting. The development and application of medical devices produced by 3D bioprinting and 3D bioprinting of tissues or their components go together hand-in-hand and are used in many medical fields and specialties.

However, I would like to review the current state of art of this burgeoning field. Bioprinting can be applied to printing of tissues using living or viable components, such collagen scaffolding, cartilage components, cells, and also to printing parts of structures, such as teeth, using artificial, man-made materials that will become embedded in bodies. Many types of components are integrated for numerous applications. Other medical applications may involve printing of devices, such as prostheses and pacemakers, which not quite embedded with tissues are nevertheless integrated with body functions and physiology, and may become an integral as well as biodegradable part of relevant tissues and organs sometime in the future.

2.2.1. Plastic and Reconstructive Surgery

This is perhaps the number one field pursuing development of bioprinting and its use. It has been making advances in the development of branches or techniques of 3D printing to manufacture a multitude of human and animal tissues. A lot of the studies are still in the experimental stage, testing 3D bioprinting of tissues and/or their components, such as skin, nasal and ear cartilage, trachea, peripheral nerves, on rats and mice [2]. Skin bioprinting is still in the experimental stage mostly because of the complexity of skin multiple layers, hair follicles and several types of glands, and the variety of cell types participating and embedded in skin composition [2,9,10]. An important aim of many studies is construction of decellularized extracellular matrix (ECM), an important scaffold and cell-proliferation stimulating environment [10], leading to faster skin wound healing [11]. Many other challenges have to be solved before skin bioprinting becomes usable in wider medicine, e.g., infections affecting skin burns that can lead to sepsis (and death) and contractures impairing function of legs and arms. Integrating cellular elements and skin appendages, such hair follicles and sweat glands has shown to be a difficult task as well [2]. So far most progress has been achieved to some extent with sweat glands [12] and with the advent of 4D printing (see below).

Craniofacial reconstruction has been quite challenging as well due to the anatomical complexities, spatial configurations and participation of multiple types of tissues and cells in this area. Reconstruction of nasal and auricular components, including cartilage and skin has been attempted using 3D bioprinting. Reconstruction of nasal cartilage has been studied using combination of natural and man-made materials [13,14]. Though a lot of progress has been made most of it is still tested in tissue models and animal trials [2]. Though many biomaterials, including cell-laden lipo-transfer collagen scaffolds appear to stimulate adipose tissues and production of growth factors [15], some of them induce rapid degradation and strong immune response to implants [16].

2.2.2. Orthopedics

Orthopedics has benefited already quite a bit from 3D bioprinting as quite a few biomaterials, such as ceramics, but also calcium phosphate and hydroxyapatite as well titanium and tantalum have been used for bone repair and reconstruction with or without bioprinting for some years (see below under Biomaterials). Overall, 3D bioprinting has proven to be well suited for orthopedics, perhaps because bone regeneration and the use of substitute materials and their production, including bioprinting has been explored for much longer than for other tissue, and perhaps because bone is more amenable to reconstruction by bioprinting. Several printing techniques have been utilized with success, such as extrusion-based technique and SLA. Variety of bioinks and cell types have been useful in promoting osteogenesis [2]. Interestingly, Amler et al. have shown difference in osteogenesis, mineralization and osteogenic differentiation among mesenchymal progenitor cells derived from different bones [17].

2.2.3. Dentistry

This is one field ideally suited for 3D bioprinting, and also the one who has been using 3D printing for long time. The practical Perceptual admission test (PAT), a part of the Dental admission test, is required by many dental schools during entrance exams. PAT evaluates candidates' spatial visualization skills as dentists need to construct teeth and put together mental images of teeth from X-rays, create and manipulate casts and fillings, and evaluate complicated 2D and 3D objects. All of this can be done by 3D printing and 3D imaging to speed up and improve the process of creating new teeth and crowns and their quality among other things. Many dental subspecialties, such as prosthodontics, oral and maxillofacial surgery, prosthetics have been utilizing and developing new techniques in 3D printing [18,19]. Faithful reproduction of teeth has proven to be of great use and help as surgical guides and in education of dentists. These are time-saving techniques, bringing care to patients faster and allowing designing of complex implants and teeth with ease in timely fashion [19].

Several techniques are both popular and very suitable for dentistry, and already described to some extent above. Stereolithography (SLA) works well for curing polymeric resin where each resin layer is dried and cured before the next layer is applied. Selective laser sintering (SLS) consists basically of laser fusing tiny particles during the object construction. It uses a wide range of materials, from ceramics to medals and polymers. Its biggest plus is production of high-density materials, however, it requires large infrastructure [20,21]. A big advantage of FDM is super-fast hardening of the thermoplastic resin upon extrusion from a nozzle (less than a minute) [19].

2.2.4. Peripheral Nerve Reconstruction

To solve the problem of the limited ability of nerves to regenerate after external injury or degenerative disease has been a major task for neurologists and neurosurgeons alike. Attempts to use nerve autografts and allografts have met with rather limited success [2].Some development of 3D bioprinted grafts from decellularized ECM and synthetic material to replace injured and/or dead peripheral nerves has been achieved [22]. Another approach for reconstruction combines the use nerve guidance conduits with stem cells [23]. These innovative venues have been in progress only in laboratories and animal trials so far and human use is still in the future. Not just 3D printing but also 3D imaging and modeling is essential to dentistry.

2.2.5. Ophthalmology

This still somewhat mysterious, though absolutely essential medical field for human health, has been developing bioprinting to treat several corneal conditions. Corneal reconstruction with 3D

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bioprinting has been helped with graft development aiming at preserving proper strength, curvature, biocompatibility and optical clarity, though so far it has not been possible to construct full-thickness cornea [2,24]. One group has been successful in construction of corneal stromal layer using collagen-based bioink embedded with primary human corneal stromal keratocytes [25].

2.2.6. Medical Devices

As it is pointed out above 3D printing of medical devices has been used with great success for years. The fusion deposition method (FDM) of printing is particularly well suited for this purpose. These devices are not a uniform set. One group include implants, prosthetics, surgical equipment, and anatomical models among other things and are built layer by layer from digital models by adding materials. Another one is represented by pharmaceuticals developed for controlled release of drugs, including quickly dissolving pills or multi-layered tablets with varying release rates. This is different from products that combine drugs with devices to provide therapeutic effect. Many such contraptions are widely used by millions of people without realizing that sophisticated 3D printing manufacturing is responsible for development of drug-eluting stents, pre-filled syringes, inhalers, and transdermal patches. These are examples of pharmaceuticals daily used by insulin-dependent diabetics, asthma patients, women using certain types of contraception, smokers or former smokers fighting their nicotine addiction [26].

2.3. Biomaterials

There is a whole slew of biomaterials available for use in 3D printing (additive manufacturing). Some of them are pure biomaterials derived from tissues, extracellular matrix and cells. They might be used as such, or they might be processed to make them more adaptable for specific use. Polymers are exquisitely suitable for 3D printing as they are versatile, easy to use and they might be derived from biological or man-made materials [1].

Natural polymers are abundant as essential components of tissues and organs and indispensable for proper functioning of living, including, our bodies [27]. Many are used in manufacturing of paper and textile goods, as food additives, and in nutraceuticals [27]. Their use in cosmetics [28] and drug delivery can be considered biomedical application [29], but do not necessarily require 3D bioprinting to be functional. Natural polymers are used together with cells directly in 3D bioprinting. Such polymers are polymeric hydrogels functioning as bioinks to encapsulate cells in the process of 3D printing of tissues, vascular/neural networks and even entire organs, the Holy Grail of organ transplantation. Their properties of water absorption and retention, biocompatibility and lack of toxicity contribute to creation of a stable environment for cells to grow, migrate, proliferate, and/or differentiate, and ultimately to continue to function as transplanted organs inside bodies. Good examples of such polymers are gelatin, alginate, fibrinogen, hyaluronic acid. They are water-soluble, all of them are widely available, relatively cheap and easy to work with [30]. These compounds form bioinks to which cells are be added before bioprinting [31,32]. Growth factors are another example of such an addition promoting regeneration and cell proliferation [33,34]. The environment provided by hydrogels enables extracellular matrix development, initial organ formation, including vascular/lymphatic/neural networks upon implantation in vivo [30]. The enormous capacity of hydrogels for water absorption is utilized in manufacturing targeted drug delivery systems where drugs are delivered in small devices that upon implantation into tissues are slowly released from the device [35].

Polylactic acid (PLA) is widely used polymer made from fermenting common sugars extracted from corn or sugar cane. PLA is suitable for manufacture of biomedical products as it is of low toxicity and biodegradable (into lactic acid), that makes it suitable for use as a component of sutures/staples and drug delivery devices which means that with time they are degraded and replaced with connective tissue after implantation into the body [36]. PLA (among many other biomaterials) has been used in healing of recalcitrant and difficult bone defects [37]. Though products such as staples and sutures are routinely manufactured and distributed among medical facilities in large quantities,

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the ease and versatility of 3D printing would be welcome by physicians such plastic surgeons with urgent needs to individualize and modify sutures and staples for their patients.

Flexible polymers, such as thermoplastic elastomers (TPE), thermoplastic polyurethane (TPU) and thermoplastic co-polyesters are widely used in 3D printing of medical supplies (and other components used in other areas) because of their durability and rubber-like features in addition to the flexibility [1,38].

Somewhat surprisingly, several metals found their way into medical field as well. Titanium has been used to construct dental and medical implants, including hip and knee replacements because it is light, biocompatible, not corrosive and integrates well with bone structures. These qualities are improved when titanium is used in alloys rather than as pure metal [39]. These alloys are also very amenable to 3D printing. Recently, titanium scaffolds were 3D bioprinted to be used for periodontal ligament regeneration [40]. Tantalum, not to be confused with titanium is used to repair hard tissues, e.g., bones through drug delivery systems [41].

2.4. Bioinks and Nanocomposite Bioinks

Bioinks are basically the substance sine qua non for bioprinting. It is a combination of biomaterials with cells, though some scientists apply the designation only to biomaterials. These biomaterials have properties suitable for bioprinting of specific tissues and for accommodation and high special distribution of specialized, or at least cells able to differentiate into such cells [32]. Hydrogels are perhaps the most important biomaterials because they facilitate and allow cell support, proliferation and differentiation. They allow hydration, oxygen and nutrient permeability, are biodegradable and biocompatible. Biomaterials in the form of nanocomposites have additional advantages over the more "traditional" biomaterials. Nanocomposites added to regular biomaterials even in small quantities enhance surface interactions, viscosity, printability, biocompatibility among other effects, and thus improve cell incorporation into biomaterials and ultimately the printability of these bioinks [32,42]. As mentioned above stereolithography (SLA) is used in their production [4]. By necessity, only a brief description of several nanocomposite bioinks follows.

Silicon based nanobiomaterials are widely used for their ability to strengthen polymeric nanocomposites. Some nanosilicates improve cell viability after printing for up to 120 days [43]. They are well suited induce osteogenesis, especially when so called Laponite nanosilicates added to cross-linked gelatin methacryloyl formed nanoengineered ionic-covalent entanglement (NICE) bioinks, Together with primary bone marrow-derived human mesenchymal stem cells (hMSCs) when printed and UV cross-linked, and incubated in a special medium without growth factors the NICE bioinks induced encapsulated hMSCs to secrete glycosaminoglycans, proteoglycans and collagen and this remodel the bioprinted scaffolds. Amazingly, transcriptome sequencing showed increased expression of numerous genes (*SOX9*, *COL1A2*, *TGFβ2*, *SMAD*, several *BMP*s) upon *in vivo* implantation of these [44].

In general, the requirements for successful bone/skeletal application of bioinks is the presence of encapsulated cells which stimulate cell proliferation and differentiation and promote vascularization (also helped with addition of vascular endothelial growth factor) [32].

Other uses for silica nanoparticles (SiNPs) have been found in bioimaging and in gene and drug delivery enabled by SiNPs regular spherical shapes and large surface area in addition to thermal and mechanical characteristics [32].

Ceramic-based nanomaterials have been shown to be of great use in tissue engineering and regenerative medicine (TERM) as they promote repair of bone voids and defects, a fairly common unwelcome event in trauma medicine and orthopedics. Their biocompatibility and osteoconductivity, together with their similarity to natural bone minerals, greater resistance to degradation than hydrogels make very suitable for use in bone repair [32]. Bioactive glass nanoparticles, belonging to the same class of nanoparticles as ceramic nanomaterial, have shown their usefulness in osteogenesis and bone tissue engineering as well [45].

Calcium phosphate (CaP) - nanoparticles is a group of related compounds that are very similar to bone and are very useful in bone regeneration. Hydroxyapatite is one of them and because of its good bioaffinity and biocompatibility and perhaps mainly because of its similarity to its natural counterpart present in the bone is exquisitely suited for use as a component in contrast agent for microcomputed tomography [32]. When incorporated with hydrogels (e.g., alginate and gelatin) the composite bioink was excellent for 3D bioprinting of bone constructs due to its good cell viability (alginate merit), structural stability (provided by gelatin} and osteoconductivity (presence of hydroxyapatite) [46].

Cellulose-based nanomaterials are derived from cellulose and include nanocellulose, cellulose nanofibrils/ nanocellulose fibrils, cellulose nanocrystals/nanocrystalline cellulose and nanocellulose blends. Nanocellulose is crystalline, high specific surface area, surface chemical reactivity and biocompatibility, to name at least some of its characteristics important in bioprinting. It mimics the fibril network of ECM so it serves as reinforcement of structures in bioprinting. This properties makes it indispensable as an additive to hydrogels, in bioprinting of cartilage, tendon, bone, skin, face (plastic surgery) and other tissues and organs [32]. Alginate, a form of cellulose-based material, when combined with nanocellulose fibrils has found its way into 3D printing of cartilage tissues [47].

3. Four-Dimensional Bioprinting

4D bioprinting appeared on the scene when it became necessary to incorporate dynamic changes in shape. This requirement led to adding the 4th dimension of time in the form of changing shape to the three existing static dimensions [48]. Five parameters are required to fulfill successful environment for 4D printing. Those are the additive manufacturing (AM) process, the printing material, the stimuli, the mode of interaction and the type of modeling [49,50]. Let's describe very briefly the merits of these five parameters. The AM process of printing allows direct manufacturing of print media from the computer without the presence of an intermediate device. A few printing methods are described in the section below. Some of the print media AKA smart materials are mentioned throughout the manuscript and also further down as well [51]. The use of a specific smart material determines what stimuli should be used to direct the path of specific transformation of the smart material [52]. The stimuli can be further characterized as physical (light, humidity, magnetic and electrical energy, temperature and UV light). Chemical stimuli or chemicals, can be further divided by their pH and may include both oxidizing substances and reducing media. Biological stimuli involve enzymes and glucose [49,53]. The mode of interaction and its mathematical modeling are the remaining two parameters. The mode of interaction varies with the type of material used and will therefore lead to modification of modeling. This will lead to determination of the time most favorable to maximize the effect of a particular stimulus on the smart material [54]

Fulfilling these conditions is of particular importance for the medical field during production of biomimetic tissues which change shape or function depending on stimuli as they appear with time. Or one might say that in the 4D world, 3D structures become dynamic and change shape or function with time and depending on the nature of stimuli [55]. The discovery and manufacturing of shape-memory materials have been transforming not only biomedicine, but also many unrelated industries, such as space, textile, sports, defense automobile manufacturing [55]. Smart designs for 4D printed structures must be pre-programmed in computer-aided design (CAD) by calculating time-dependent shape-changes or deformations of so called smart materials able to change their geometry in response to external stimuli [56]. Such materials are capable of self-assembly, self-healing, remembering their shapes, self-reproduction [48,57]. All these properties make these materials ideal to be used in biomedical fields and in particular in TERM, drug delivery and, in the future, in creating living organs suitable for transplantations [35,58].

3.1. Printing Methods for Shape-Shifting Scaffolds [58]

Just as with 3D printing several techniques were developed, or modified from 3D versions for 4D printing. Several types of extrusion-based printing found their home in 4D bioprinting: fused deposition modeling (FDM) (see above under 3D printing), direct ink writing (DIW), inkjet printing, SLA, and digital light processing [48] are the most commonly used modalities.

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FDM is particularly suitable for printing of thermoplastic polymers, not only because of the fast recovery of shape of compressed scaffolds but because of induction of shape change in seeded mesenchymal stem cells [59].

This would initiate proliferation and differentiation of such cells and potentiate if not initiate TERM as well.

Direct ink writing has been found useful for fabrication of self-healing scaffolds, using urethane diacrylate/ polycaprolactone for manufacturing patches for vascular repair [60]. The use of visco-elastic liquid- or paste-like inks is advantageous in heat-free environment [61]. Some examples of the many materials suitable for this type of printing include nanocomposite polymeric solutions, hydrogels, resins and several ceramic-based materials. Once printed the products solidify through a temperature change or using a crosslinking/gelatin mechanism [58]. This method has proven to be very useful for fabrication of vascular patches using highly stretchable scaffolds that have shape memory and self-healing ability [60].

Similar to direct ink, printing Cryo-3DP transforms liquid printed forms manufactured from hydrogels and other biomaterials into solid, stable structures used for repair of bone defects. The liquid material is printed at -10^{o}C and compressed to a very small volume which expands when exposed to near-infrared irradiation [62].

Extrusion-based bioprinting, another form of FDM, is well suited for printing of bioinks carrying cells. Care must be taken to keep the viscosity not too high so the cells remain viable extrusion. This is accomplished by careful increase of applied shear rate and the printing speed among other parameters [58].

Inkjet-based printing is commonly used to print both biological and non-biological materials. It was originally designed for fabrication of bilayers of gelatin-based materials. When immersed in aqueous media the scaffolds would self-roll into microtubules mimicking small blood vessels. These structures have the ability to support the implantation and proliferation of endothelial cells [63]. In addition, droplet-based printing was shown to produce self-assembling of cells into structure capable of self-assembly in tissues when stimulated by light [64].

As mentioned above stereolithography (SLA) is used in manufacturing of bioinks and in dentistry. UV laser is used to solidify liquid photopolymer in layers where each resin layer is dried and cured before the next layer is applied [4]. As the resin is exposed to air, oxygen may inhibit curing and this may result in incomplete crosslinking and overhanging of the material. In addition, the resin-recoating in the SLA process is rather slow [65]

Digital light processing or DLP resembles SLA but because of its ability to solidify a layer of photopolymer resin during single exposure it is much faster. This is achieved by inclusion of so called digital micromirror device (DMD) where the micromirrors rotate to direct the light to cure the resin layer in one shot. Both SLA and DLP are nozzle free systems with fast printing, high printing resolution and acceptable cell viability [65].

Laser-assisted bioprinting (LAB) is another laser-utilizing printing technique. It is a nozzle-free method using a laser pulse ejecting bioink layer in droplets that contain cells. This is a method with high printing resolution and preservation of cell viability [66].

3.2. Smart Biomaterials

The main and most significant contribution of 4D printing lies in the use of stimuli-responsive biomaterials enhanced by stimuli activating transformation of the printed structure during the post-printing phase. This leads to structural or functional change [58]. Shape memory polymers (SPMs) are perfectly suited for this process (see below). For example, such constructs can be compressed and implanted during a minimally invasive procedure only to inflate to its original and functional size once *in situ* [62]. For example, nitinol, a shape memory alloy of nickel and titanium, found its way into surgical instruments and implantable medical equipment. Their compressibility before implantation and heat- induced expansion back to original shape upon implantation of insertion into living tissue has been utilized in many areas, such as orthopedics, vascular stents, and orthodontics [67].

3.2.1. Shape Memory Polymers

These polymers maintain temporary shape and come back to their permanent shape when exposed to external stimuli (heat, magnetic field, stress and light). That can happen under certain conditions. For example, when the polymer is exposed to a melting temperature it is deformed and acquires its “permanent” shape. At a temperature below its melting temperature, the material acquires its “temporary” shape [65]. in addition to their shape-morphing capabilities. Because some, but not all, are made from plant materials these polymers are biocompatible and enable cell viability and growth during and after bioprinting. Synthetic SMPs may actually by cytotoxic, especially during manufacturing involving high temperature and organic solvent [65].

3.2.2. Shape Morphing Hydrogels

In general, hydrogels are water-containing polymers (therefor the term hydrogel). The swell or shrink with the amount of water content, or change their shape with temperature changes. They are biocompatible and biodegradable, they resemble extracellular matrix and provide good supportive environment for the proliferation and maintenance of embedded cells [65].

3.2.3. Smart Composite Biomaterials

Sometimes addition of other biomaterials is required to overcome certain disadvantageous properties, e.g., limited shape morphing, poor mechanical strength, insufficient printability and low biocompatibility. Many times, the addition of only small amounts of other biomaterials (e.g., micro particles), especially those called nano-biomaterials like nanofibers (see above) can induce desirable shape changes [68].

3.3. Stimuli Involved in Shape Transformation

As already mentioned, those stimuli are necessary to transform shape into its desirable form post-fabrication, meaning after implantation and usually applied to TERM. As, under the previous subheading, there are quite a few items in this category, for the sake of the purpose of this paper only a few will be described.

Hydration is one of the most, if not the most common form of inducing shape-change, particularly well-, suited for application in our bodies containing a large percentage of water. Hydrogels suit this purpose very well: they are hydrophilic, three-dimensional crosslinked polymers. They absorb a lot of water while remaining stable, respond to humidity and temperature and exhibit memory of previous shapes. Hyaluronic acid and gelatin are two commonly used examples of hydrogels. Many hydrogels are quite biocompatible and non-toxic, and even being present in our bodies is substantial quantities, e.g., hyaluronic acid [58].

Temperature plays an important role facilitating shape transformation in TERM processes. It is easily applied in TERM conditions. Shape memory polymers respond well to temperature changes. Such polymers can be compressed at high temperature of 65^{o}C and expand to their original shape at lower temperature [58]. Polymers are first compressed to 30% of its original size at temperature lower than body temperature and expanding to their original shape at body temperature of 37^{o}C are particularly useful to be used for treating bone defects [69].

Light does induce changes in shape, such as uniform cell alignment and tissue maturation, especially with the application of NIR laser [70].

At times multiple stimuli are used to trigger shape changes multiple times but this is beyond the scope of this review [58].

The use of cell-containing bioinks enables encapsulation of cells in the scaffold, as well as several cell-seeding technologies. Cell can be incorporated into scaffolds post-fabrication, post-printing but before pre-4D stimulus is applied. Cell incorporation done post-fabrication can be accomplished with 3D printing technique and scaffolds with cell implanted this way are useful for drug delivery, they can function as biomolecules or biomedical devices [58]. In the second strategy cell seeding is done between printing and pre-4D stimulus. This is useful if the printing would lead to cell damage and would decrease cell viability. The third form of fabrication where cells are incorporated into bioinks pre-printing has the advantage of delivering into desired place during delivery, but it requires highly cell friendly bioinks [58].

The possibilities of 4D printing use are many and are expanding. Components for tissue repair and regeneration are similar to those used in 3D printing: scaffolds (or matrix) providing support to cells (usually stem cells or primary cell cultures) and signaling molecules (growth factors and cytokines, mechanical, physical and electrical stimuli. Cells supported by matrix and stimulated in most cases by multiple stimuli can grow and differentiate simulating in vivo situation. Smart scaffolds should provide support as close as similar to in vivo matrix support. Drug delivery has a place in 4D bioprinting as well where the use of smart hydrogels facilitates release of drugs upon the presence of external triggers, such as temperature, pH change, electrical and magnetic fields. Light and drug concentration [35,71]. Obviously, 4D printing of organs suitable for transplantation is still some years away, though the usefulness and applicability of 4D printing in TERM and creating of organoids, the “babies” of full-fledged organs, represent significant steps forward.

3.4. Smart Design

Smart design is necessary to properly direct these smart changes. Because different smart biomaterials undergo changes taking different pathways and exhibit distinct changes and variations among themselves, bioprinting of different biomaterials, each exhibiting different properties and outcome should have distinct printing parameters that would accomplish desired outcome [65]. Further discussion would be beyond the scope of this review.

3.5. Properties of Bioinks Used in 4D Bioprinting

Though I am sure this would be of interest to many readers, elaboration of properties of bioinks is again beyond the scope of this review. Suffice to list some desirable properties of these bioinks and a comprehensive review of 4D bioprinting by Lai et al. Those properties taken into consideration are: biocompatibility, stimuli-responsiveness, biodegradability, mechanical properties, cells and their viability [65].

3.6. Current Applications of 4D Bioprinting

Though this field is similar to 3D bioprinting applications, the development of 4D applications brings the emergence of dynamic, newly created constructs and materials approximating and mimicking dynamics of living tissues and structures *in vivo*. A few examples of areas where 4D bioprinting has been shown of use in medicine.

3.6.1. Skin

As pointed out above in*3D bioprinting in biomedical applications*, skin bioprinting has been very challenging and not very successful. 4D bioprinting offers several promising solutions. One consists of fabricated dynamic skin grafts. Another effort produced 4D bioprinted skin capable of changing shape (smart shape skin) adjusting to changes in geometry of healing skin wounds. These new methodologies are utilized both *in vitro* and *in vivo* (*in situ*) environment [72]. These new models are characterized by cooperation and interactions between skin cells and ECM, an important part of physiological processes in all organs.

3.6.2. Bone

3D bioprinting has been useful in enhancing bone regeneration and repair of bone defects for some years. The use of shape memory polymers and shape morphing hydrogels enhanced maturation of bone scaffolds [73]. Mesenchymal stem cells when cultured in osteogenic medium can be used in oxidized and methacrylated alginate scaffolds for bone regeneration by undergoing osteogenic differentiation and promoting mineralization [74]. SMPs are used in bone 4D bioprinting in building shape memory scaffolds. Their change in shape also leads to adjustment to irregular bone defects and thus to improved bone regeneration. Bone regeneration is further enhanced by the addition of growth factors and minerals [65].

3.6.3. Cartilage

Progress in creating cartilage has been achieved by 4D bioprinting of cell-laden self-folding scaffolds, Such scaffolds are printed the DLP methodology [75]. DLP-created hydrogel bilayer responds to water by folding into a tube-like structure, ready to be used e.g., as an implant of trachea (so far tried in a rabbit).

3.6.4. Vasculature

Blood vessels have been constructed by 3D bioprinting for a while. 4D bioprinting improves the precision and flexibility with better control of (small) diameters and complexity of vasculature, especially in the cases of small vessels [65].

3.6.5. Medical devices

4D bioprinting resulted in adding many favorable dynamic properties to devices such as stents, occluders, microneedles, smart 3D-cell engineered microenvironments, drug delivery systems, wound closures, and implantable medical devices, some of them already mentioned in this paper above. Besides using smart design, shape memory biomaterials and smart stimuli, the contribution of machine learning and AI will transform the field 4D bioprinting even more [76].

4. Future Directions

3D printing came to life in 1984 as an additive manufacturing method of three-dimensional objects directed by digital means. Tri-dimensional bioprinting is becoming indispensable in manufacturing individualized dental and orthopedic prostheses. In addition, cultures of stem cells together with their development into distinctly differentiated types of cells was one of the first steps leading to *in vitro* formation and cultivation of complex tissues, including constructs combining cells with growing on scaffolds of collagen and extracellular matrix. The possible applications of these man-made tissue prototypes became more realistic with the advent of 3D bioprinting. Preparation of complex tissues for implantation to enhance wound healing and regeneration is coming of age with 3D bioprinting. This relatively recent modality brings the aim of growing organs in vitro for transplantation closer to reality. This is not the final step in bioprinting and organ creation as so called 4D bioprinting enables shape changes with stimulation in time. This advancement represents a tremendous step forward in creating and assembling organs for transplantation in humans. This would greatly help many very ill people spending less time on waiting list for an organ donor (who is usually dead) and would represent one or more goals in precision medicine – a brand new organ ready for the recipient who, because of shorter time on the waiting list and because the organ would be custom-made would have a better chance for longer and qualitatively better life than such patients have today. Future 4D bioprinting as it would become more adaptable to *in vivo* conditions and would be able to create organs using the recipient's cells to relegate graft rejection to the past. That would be a significant improvement in terms of patient recovery and long-term prognosis as immunosuppressive therapy necessary today for the transplant to be accepted by the new host would not be needed anymore, or at least in lower doses.

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References

1. Iftekar, S.F., et al., *Advancements and limitations in 3D printing materials and technologies: a critical review.* Polymers, 2023. **15**(2519).
2. Mirsky, N.A., et al., *Three-dimensional bioprinting: a comprehensive review for applications in tissue engineering and regenerative medicine.* Bioengineering, 2024. **111**(777).
3. Hull, C.W., *Apparatus for production of three-dimensional objects by stereolithography* G. Patents, Editor. 1986.

4. Huang, J., Q. Quin, and J. Wang, *A review of stereolithography: processses and systems.* Processes, 2020. **8**(1138).
5. Kuang, X., et al., *Grayscale digital light processing 3D printing for highly fubctionally graded materials.* Sci Adv, 2019. **5**.
6. Dumpa, N.R., S. Bandari, and M.A. Repla, *Novel gastroretentive floating pulsatile drug delivery systemproduced via hot-melt extrusion and fused deposition modeling 3D printing.* Pharmaceutics, 2020. **12**(52).
7. Kaierle, S., et al., *Development of a laser ablation model and a disposable 3D-printed tool head for weakening thick steel sheets for the deflagration of hazardous substances.* CIRP J Manuf Sci Technol, 2019. **26**: p. 94-99.
8. Zhang, Q., et al., *Multiphoton polymerization using upconversion nanoparticles for adaptive high-resolution 3D printing*, in *Advanced fabrication technologies for micro/nano optics and photonics*, G. von Freymann, E. Blasco, and D. Chanda, Editors. 2023, SPIE: Bellingham WA, USA. p. 27.
9. Weng, T., et al., *3D bioprinting for skin tissue engineering: Current status and perspectives.* J Tissue Eng, 2021. **12**.
10. Alawi, S.A., et al., *3D bioprinting in plastic and reconstructive surgery. Current concepts, progress, and clinical application.* Eur J Plast Surg, 2023. **46**: p. 833-843.
11. Zhang, D., et al., *3D-bioprinted biomimetic multilayer implants comprising microfragmented adipose extracellular matrix and cells improve wound healing in a murine model of full-thickness skin defects.* ACS Appl Mater Interfaces, 2023. **15**: p. 29713-29728.
12. Huang, S., et al., *3D bioprinted extracellular matrix mimics facilitate directed differentiation of epithelial progenitors for sweat gland regeneration* Acta Biomater, 2016. **32**: p. 170-177.
13. Ruiz-Cantu, L., et al., *Multi-material 3D bioprinting of porous constructs for cartilage regeneration.* Mater Sci Eng C Mater Biol Appl, 2020. **109**(110578).
14. Zielinska, D., et al., *Combining bioengineered human skin with bioprinted cartilage for ear reconstruction.* Sci Adv, 2023. **9**(eadh1890).
15. Conci, C., et al., *Tissue engineering and regenerative medicine strategies for the female breast.* J Tissue Eng Regen Med, 2020. **14**: p. 369-387.
16. Grounds, M.D., *Obstacles and challenges for tissue engineering and regenerative medicine: Australian nuances.* Clin Exp Pharmacol Physiol, 2018. **45**: p. 390-400.
17. A.K., A., et al., *Comparison of the translational potential of human mesenchymal progenitor cells from different bone entities for autologous 3D bioprinted bone rafts.* Int I Mol Sci, 2021. **22**(796).
18. Kaur, N., D. Grover, and G. Kaur, *Future dental device 3-dimensional printing approach in dentistry.* JP Int J Maxillofac Imaging, 2021. **7**: p. 37-47.
19. Chakravorty, S., et al., *3D printing: opening new horizons in dentistry.* J Pharm Bioallied Sci, 2024. **16**: p. S8-S10.
20. Jeong, M., et al., *Materials and applications of 3D printing technology in dentistry: an overview.* Dent J, 2023. **12**(1).
21. Yang, J., et al., *Selective laser sintering versus conventinal lost-wax casting for single metal coping: a systematic review and meta-analysis.* J Prosthet Dent, 2022. **128**: p. 897-904.
22. Min, K., et al., *Three-dimensional microfilament printing of a decellularized extracellular matrix (dECM) bioink using a microgel printing bath for nerve graft fabrication and the effectiveness of dECM graft combined with a polycaprolactone conduit.* ACS Appl Bio Mater, 2022. **5**: p. 1591-1603.
23. Georgiou, M., et al., *Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve.* Biomaterials, 2015. **37**: p. 242-251.
24. Fuest, M., et al., *Prospects and challenges of translational corneal bioprinting.* Bioengineering, 2020. **7**(71).
25. Duarte Campos, D.F., et al., *Corneal bioprinting utilizing collagen-based bioinks and primary human keratocytes.* J Biomed Mater Res Part A, 2019. **107**: p. 1945-1953.
26. Patel, R., et al., *3D printing chronicles in medical devices and pharmaceuticals: tracing the evolution and historical milestones.* J Biomat Sci, Polymer Ed, 2024. **35**: p. 2723-2766.
27. Silva, A.C.Q., et al., *Natural polymers-based materials: a contribution to a greener future.* Molecules, 2022. **27**(94).
28. Bukhari, S.N.A., et al., *Hyaluronic acid, a promising skin rejuvenating biomedicine: A review of recent updates and pre-clinical and clinical investigations on cosmetic and nutricosmetic effects.* Int J Biol Macromol, 2018. **120**: p. 1682–1695.
29. Tong, X., et al., *Recent advances in natural polymer-based drug delivery systems.* React Funct Polym, 2020. **148**: p. 104501.
30. Liu, F., et al., *Natural polymers for organ 3D bioprinting.* Polymers, 2018. **10**(1278).
31. Fatimi, A., et al., *Natural hydrogel-based bio-inks for 3D bioprinting in tissue engineering* Gels, 2022. **8**(179).
32. Cai, Y., et al., *Nanocomposite bioinks for 3D bioprinting.* Acta Biomaterialia, 2022 **151**: p. 45-69.
33. Zhang, X. and Y. Zhang, *Tissue engineering applications of three-dimensional bioprinting.* Cell Biochem Biophys, 2015. **72**: p. 777-782.
34. Zhang, Y.S., et al., *3D bioprinting for tissue and organ fabrication.* Ann Biomed Eng, 2017. **45**: p. 148-163.
35. Bordbar-Khiabani, A. and M. Gasik, *Smart hydrogels for advanced drug delivery systems.* Int J Mol Med, 2022. **23**(23665).

36. Liu, Z., et al., *A critical review of fused deposition modeling 3D printing technology in manufacturing polyactic acid parts.* Int J Adv Manuf Technol, 2019. **102**: p. 2877-2889.
37. Rodriguez-Merchan, E.C., *Bone healing materials in the treatment of recalcitrant nonunions and bone defects.* Int J Mol Sci 2022. **23**: p. 3352.
38. Leon-Calero, M., et al., *3D printing of thermoplastic polymers: role of the chemical composition and printing parameters in the production of parts with controlle energy absorption and damping capacity.* Polymers, 2021. **13**: p. 3551.
39. Marin, E. and A. Lanzutti, *Biomedical applications of titanium alloys: a comprehensive riview.* Materials, 2023. **17**: p. 114.
40. Lee, L.L., et al., *Bioprinting on 3D printed titanium scaffolds for periodontal ligament regeneration.* Cells, 2021. **10**: p. 1337.
41. Hua, L., et al., *3D-printed porous tantalum: recent application in various drug delivery systems to repair hand tissue defect.* Expert Opin Drug Deliv, 2021. **18**: p. 625-634.
42. Gaharwar, A.K., N.A. Peppas, and A. Khademhosseini, *Nanocomposite hydrogels for biomedical applications.* Biotechnol Bioeng, 2014. **111**: p. 441-453.
43. Chimene, D., et al., *Nanoengineered ionic–covalent entanglement (NICE) bioinks for 3Dbioprinting.* ACS Appl Mater Interfaces 2018. **10**: p. 9957-9968.
44. Chimene, D., et al., *Nanoengineered osteoinductive bioink for 3D bioprinting bone tissue.* ACS Appl Mater Interfaces, 2020. **12**: p. 15976-15988.
45. Leite, A.J., et al., *Bioplotting of a bioactive alginate dialdehyde-gelatin composite hydrogel containing bioactive glass nanoparticles.* Biofabrication, 2016. **8**(3).
46. Wüst, S., et al., *Tunable hydrogel composite with two-step processing in combination with innovative hardware upgrade for cell-based three-dimensional bioprinting.* Acta Biomaterialia, 2014. **10**: p. 630-640.
47. Markstedt, K., et al., *3D bioprinting human chondrocytes with nanocellulose–alginate bioink for cartilage tissue engineering applications.* Biomacromolecules, 2015. **16**: p. 1489-1496.
48. Naniz, M.A., et al., *4D printing: a cutting-edge platform for biomedical applications.* Biomed Mater, 2022. **17 (6)**.
49. Mierke, C.T., *Bioprinting cells, organoids and organs-on-a-chip together with hydrogels improves structural and mechanical cues.* Cells, 2024. **13**(1638).
50. Momeni, F., X. Liu, and J. Ni, *A review of 4D printing.* Mater Des, 2017. **122**: p. 42-79.
51. Haleem, A., et al., *3D printing applications in bone tissue engineering.* J Clin Orthop Trauma, 2020. **11**: p. S118–S124.
52. Sajjad, R., et al., *A review of 4D printing—technologies, shape shifting, smart polymer based materials, and biomedical applications.* Adv Ind Eng Polym Res, 2024. **10**: p. 20-36.
53. El-Husseiny, H.M., et al., *Smart/stimuli-responsive hydrogels: cutting-edge platforms for tissue engineering and other biomedical applications.* Mater Today Bio, 2022. **13**: p. 100186.
54. Ahmed, A., et al., *4D printing: fundamentals, materials, applications and challenges.* Polymer, 2021. **228** p. 123926.
55. Khalid, M.Y., Z.U. Arif, and W. Amed, *Four-dimensional (4) printing: technological and manufacturing renaissance.* Macromol Mater Eng, 2022. **307**(8).
56. Khoo, Z.X., et al., *3D printing of smart materials: a review on recent progresses in 4D printing.* Virtual Phys Prototyp, 2015. **10**: p. 103-122.
57. Ali, M.H., A. Abilgaziyev, and D. Adair, *4D printing: a critical review of current developments, and future prospects.* Int J Adv Manuf Technol, 2019. **105**: p. 701-717.
58. Kalogeropoulou, M., et al., *4D printed shape-shifting biomaterials for tissue engineering and regenerative medicine applications.* Biofabrication, 2024. **16**(2).
59. Hendrikson, W.J., et al., *Towards 4D printed scaffolds for tissue engineering: exploiting 3D shape memory polymers to deliver time-controlled stimulus on cultured cells.* Biofabrication, 2017. **9**(031001).
60. Kuang, X., et al., *3D printing of highly stretchable, shape-memory, and self-healing elastomer toward novel 4D printing.* ACS Appl Mater Interfaces, 2018. **10**(7381).
61. Lewis, J.A., *Direct ink writing of 3D functional materials.* Adv Funct Mater, 2006. **16**(2193): p. 2193-2204.
62. Wang, C., et al., *Advanced reconfigurable scaffolds fabricated by 4D printing for treating critical-size bone defects of irregular shapes.* Biofabrication, 2020. **12**(045025).
63. Cui, C.X., et al., *4D printing of self-folding and cell-encapsulating 3D microstructures as scaffolds for tissue-engineering applications.* Biofabrication, 2020. **12**(4): p. 045018.
64. Booth, M.J., et al., *Light-patterning of synthetic tissues with single droplet resolution.* Sci Rep, 2020. **7**: p. 9315.
65. Lai, J., et al., *4D bioprinting of programmed dynamic tissues.* Bioact Mater, 2024. **37**: p. 348-377.
66. Roversi, K., et al., *Bioprinting of adult dorsal root ganglion (DRG) neurons using laser-induced side transfer (LIST).* Micromachines, 2021. **12**(865).
67. Alipour, S., et al., *Nitinol: from historical milestones to functional properties and biomedical applications.* Proc Inst Mech Eng H, 2022. **236**: p. 1595-1612.

 doi:10.20944/preprints202412.1004.v1

68. Wang, M. and Q. Zhao, *Biomedical composites* in *Reference Module in Biomedical Sciences: Encyclopedia of Biomedical Engineering*. 2019, Elsevier: The Netherlands. p. 34-52.
69. Miao, S., et al., *Four-dimensional printing hierarchy scaffolds with highly biocompatible smart polymers for tissue engineering aApplications.* Tissue Eng C, 2016. **10**(952).
70. Wang, Y., et al., *4D printed cardiac construct with aligned myofibers and adjustable curvature for myocardial regeneration.* ACS Appl Mater Interfaces, 2021. **13**(12746).
71. Municoy, S., et al., *Stimuli-responsive materials for tissue engineering and drug delivery.* Int J Mol Sci, 2020. **21**(4724).
72. Chaudhry, M.S. and A. Czekanski, *In-situ bioprinting of skin - a review.* Bioprinting, 2923. **31**(e00271).
73. Wan, Z., et al., *Four-dimensional bioprinting: current developments and applications in bone tissue engineering.* Acta Biomater, 2019. **101**: p. 26-42.
74. Ding, A., et al., *4D biofabrication via instantly generated graded hydrogel scaffolds.* Bioact Mater, 2020. **7**: p. 324-332.
75. Kim, S.H., et al., *4D-bioprinted silk hydrogels for tissue engineering.* Biomaterials, 2020. **260**(120281).
76. Yarali, E., et al., *4D printing for biomedical applications.* Adv Mater, 2024. **36**(e2402301).