Recent Advancements and Trends in 3D Printing for Biomedical Applications

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Abstract

3D printing has been applied to multiple areas since 1980. Biomedical applications have grown significantly and become the mainstream of 3D printing applications. In this review, we elucidated the publication distribution of biomedical 3D printing using the CAS Content Collection. From 2010 to 2021, journal and patent publications grew steadily, except for the decline in patent publications in 2021. In general, journal publications are higher than patents. There are approximately 90 countries participating in the 3D printing application in this field. We are reporting the publication distributions related to the following categories: (1) four major techniques: powder bed fusion, extrusion, jetting, and photopolymerization; (2) substances: polymers and inorganic substances; (3) biomedical fields: tissue/organs, orthopedic/prosthetic, pharmaceutical, and others. We investigated the correlation between these search terms and revealed the co-occurrence of the concepts appearing in publications related to 3D printing in biomedical applications. This review provides an overview of the current advancement and trends of 3D printing applications in the biomedical area.

History of 3D Printing

The earliest idea of 3D printing emerged in 1980s. It started with Hideo Kodama's first 3D printing patent application in 1980, then Charles Hull's invention of stereolithography (SLA) in 1983, Carl Deckard's selective laser sintering (SLS) in 1987 and the commercialized 3D printer in 1988, and Scott and Lisa Crump's fused deposition modeling method (FDM) in 1989. These techniques paved the way for advanced technology development and multidisciplinary applications.[1] 3D printing facilitates the concept model development with high customizability and rapid prototyping. Nowadays, 3D printing technology is utilized in various fields ranging from food, construction, automotive, and electronics to medical industry.

General Scope

For our report, we focus on the biomedical applications using 3D printing techniques to provide insights into the development of this field. Our data was collected from the CAS Content Collection, which covers publications in more than 50,000 scientific journals from around the world in a wide range of disciplines, 62 patent authorities, and 2 defensive publications (Research Disclosures and IP.com). Based on our search query (See Supporting Information), 22,000 documents were selected, and we further analyzed these documents with different search criteria.

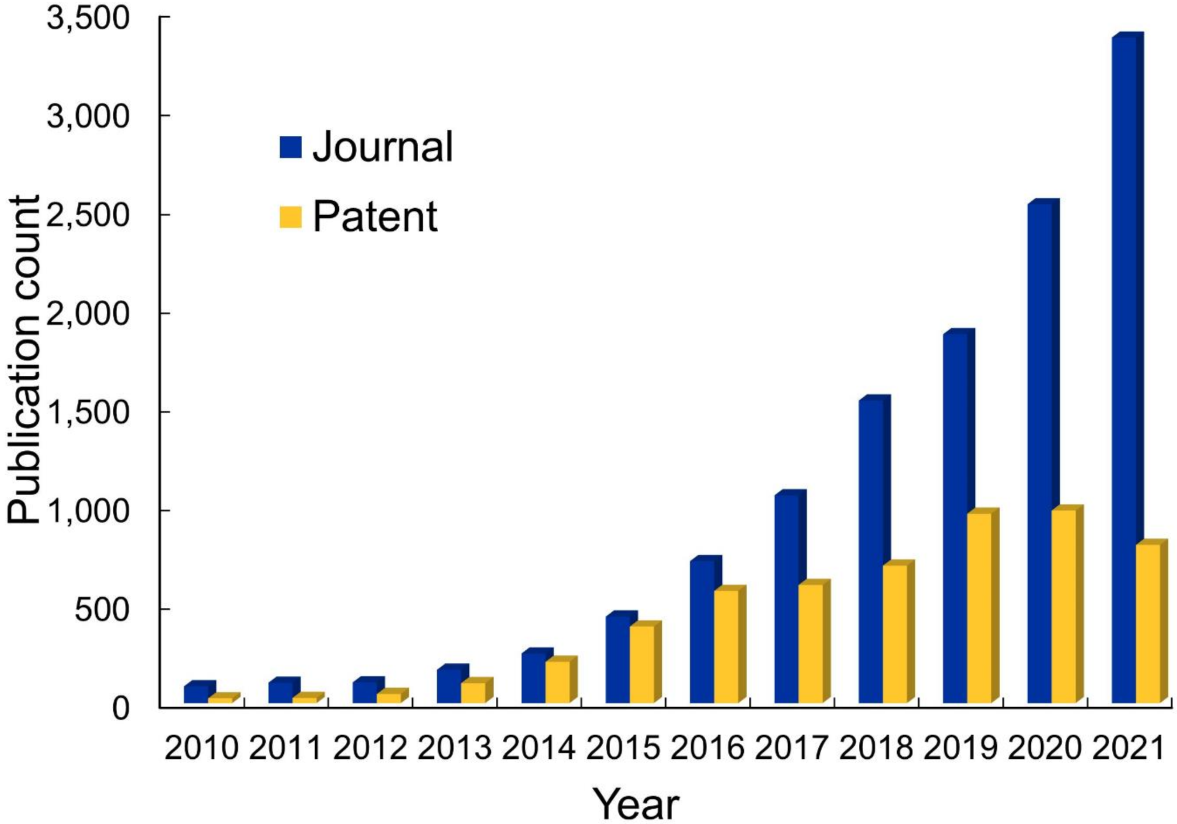


Figure 1. Annual trends of journal and patent publications for biomedical applications of 3D printing

The publications for journals and patents from 2010 to 2021 for biomedical applications of 3D printing is around 14,500 and 5,600, respectively. The publication trends feature a steady growth, as shown in Figure 1. After 2014, the publication rapidly increased in both journals and patents. Compared to the number of granted patents, the number of journal publications increased steadily after 2017, and the difference became 2500 in 2021. The innovation of biomedical application using 3D printing techniques is blooming, and the commercialization of novel ideas also increases steadily despite a trivial decline in 2021 for patent publication.

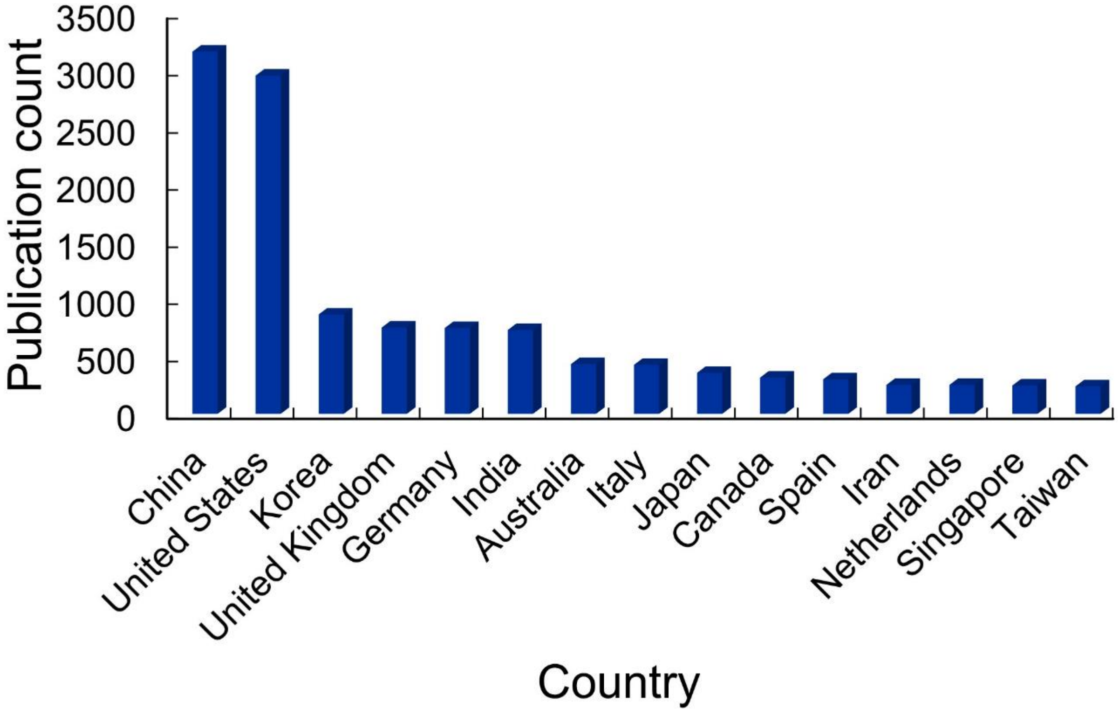


Figure 2. Top 15 countries and regions in journal publication volume related to biomedical applications of 3D printing

The top countries contributing to journal publications are shown in Figure 2. The United States and China are the leaders in journal publications, followed by Korea, the United Kingdom, Germany, and India contributing to this field with significant amounts of publications. According to our data collection, 90 countries/regions have gotten involved in this field with published journals. This data demonstrates the importance of 3D printing techniques for biomedical applications around the world.

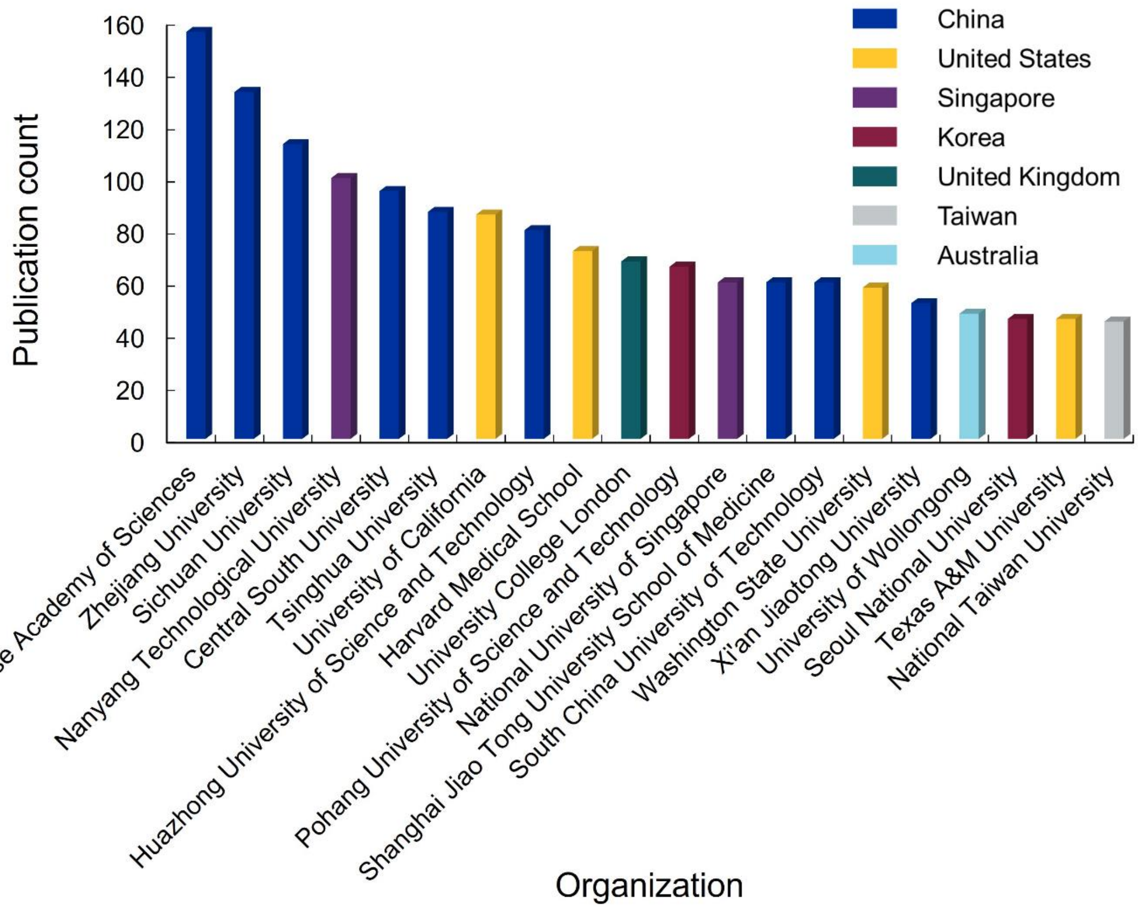


Figure 3. The 20 most productive institutes in journal publication related to biomedical applications

Among the top 20 institutes for journal publications (Figure 3), Chinese institutes are the main contributor. The Institutes from the United States, Korea, and Singapore also made remarkable contributions to the journal publications.

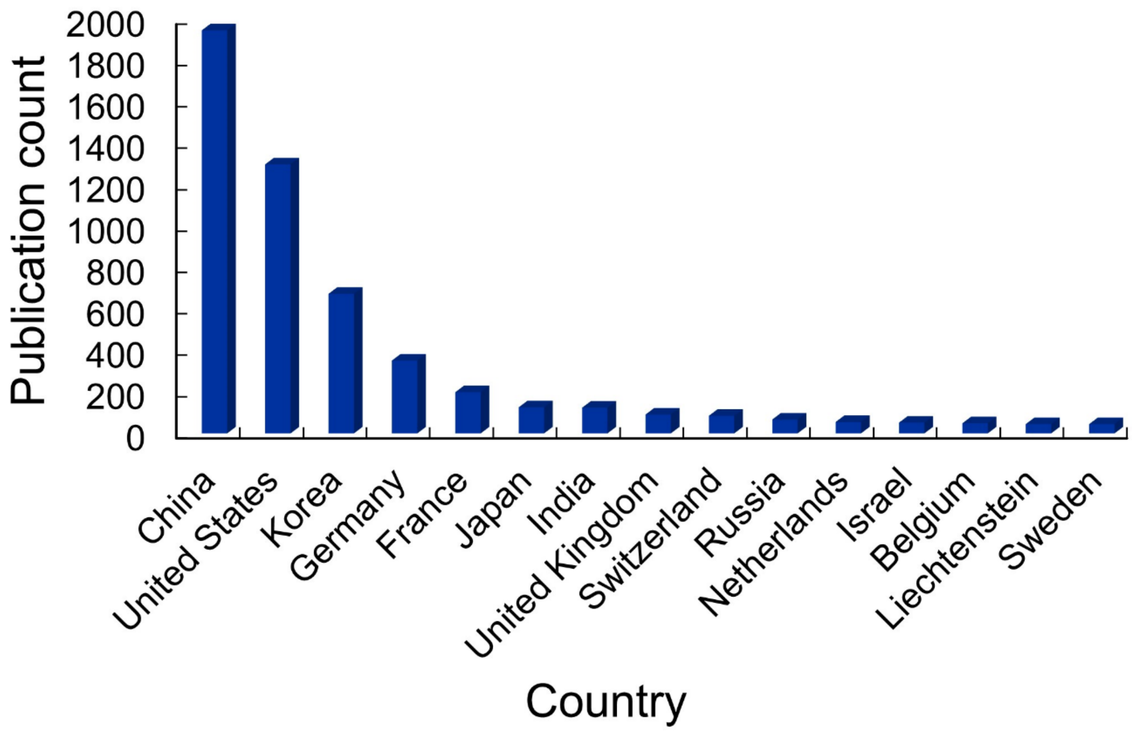


Figure 4. Top 15 countries and regions in patent publication volume related to biomedical applications of 3D printing

The number of patent publications from different countries and institutions are given in Figures 4 and 5 respectively. As indicated, China and the United States lead the publication trend of patents, followed by Korea and Germany. There are 59 countries/regions which contributed to the patents in this field.

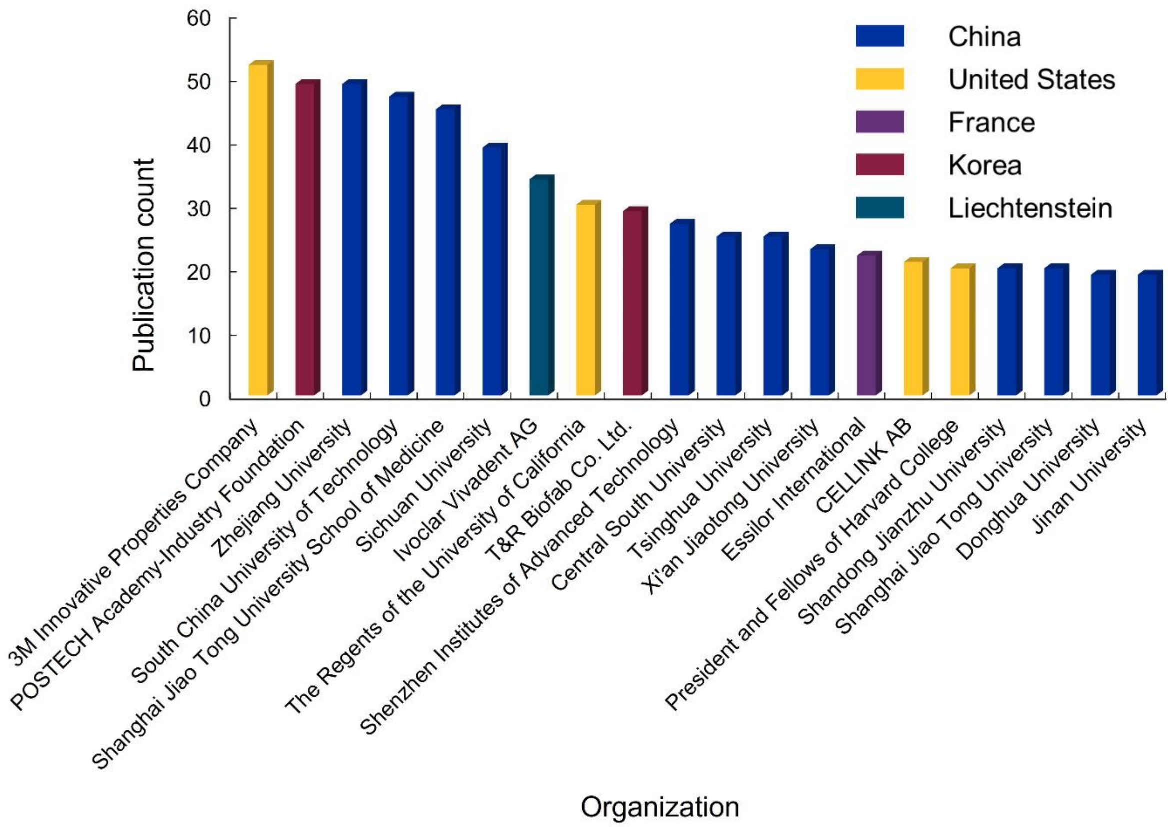


Figure 5. Top 20 patent assignees for patent publications related to biomedical applications

From the companywide aspect, Figure 5 shows that 3M, the United States-based company, has been granted the most patents in all time, followed by Ivoclar Vivadent AG in Liechtenstein, T&R Biofab Co. Ltd. in Korea, Essilor International in France, and CELLINK AB in the United States. As academic assignees, China contributed the most, followed by the United States and Korea.

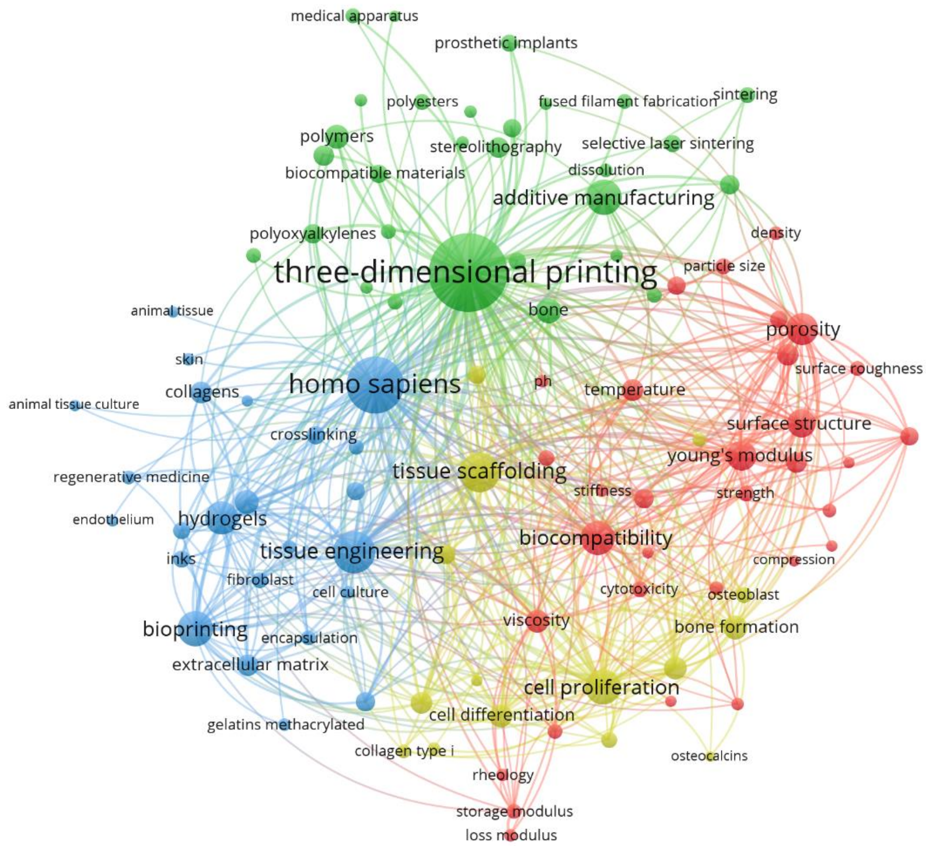


Figure 6. Co-occurrence of top 100 concepts appearing in publications related to 3D printing in biomedical applications

To further shed light on the status of development in biomedical applications of 3D printing, we have also analyzed the prevalence and connections between different concepts. The results are shown in Figure 6, where the size of a node reflects the number of times the corresponding concept occurred in the literature, lines between every two nodes denote co-occurrences in the same publication, and distances between nodes indicate the frequencies at which the concepts co-occurred. Concepts with most frequent co-occurrence are grouped into the same color: green nodes depict concepts related to 3D printing technologies and materials, blue and yellow nodes represent biomedical-related concepts, whereas red nodes correspond to characterizations and properties of 3D printed biomaterials. Several observations can be made from the diagram: (1) the prevalence of hydrogel materials in bioprinting technology is illustrated by the short distance between the concepts “hydrogels” and “bioprinting”; (2) concepts related to 3D printing techniques (except “three-dimensional printing” and “additive manufacturing”) are small and have few connections (co-occurrences) with other concepts, suggesting that 3D printing methods studies have been much isolated from research involving applications or properties of printed materials; (3) the prevalence of materials characterization concepts such as “porosity”, “surface structure”, “Young’s modulus” and their connections with “bone”, “bone formation”, “tissue scaffolding”, etc. indicate the significance of related studies.

In the following sections, we will display further publication analysis upon different search queues.

3D Printing Technologies and Their Publication Trends in Biomedical Application

First, we investigated four major printing technologies: (1) powder bed fusion; (2) jetting; (3) extrusion; (4) photopolymerization. A brief introduction of each major 3D printing technology is shown below.

Powder bed fusion

In general, the powder materials are sprayed evenly to create a thin layer for the laser or electron beam to melt the particles, followed by the rapid solidification for each layer of the 3D printed product. Selective laser sintering (SLS) and electron beam melting (EBM) are two of the common techniques applied to this 3D printing method. Materials like ceramics, thermoplastic particles, metal powders, alloys, plasters, and composites [2, 3, 4] are commonly used with this method.

Jetting

The concept of jetting is based on the regular inkjet printer. The liquid materials are injected as droplets through nozzles and quickly solidify to form a layer of material based on the path of computer-aided design (CAD). Materials in fluid phase or suspension type like polymers, nanoparticles, metals, ceramics, and bio-related materials are applicable to this method.[5]

Extrusion

Extrusion uses a nozzle to deposit the heated material layer by layer on the path determined by CAD software to build up the 3D product. Solid materials like thermoplastics, for instance, polyamides, polylactic acid, acrylonitrile butadiene styrene copolymer (ABS) are often used with this method.[6]

Photopolymerization

One typical example of photopolymerization-based 3D printing is stereolithography (SLA). SLA uses a laser to selectively cure the photopolymer from top to bottom, layer by layer while lowering the platform inside the liquid resin tank. This technology further results in biocompatible products using biocompatible or biodegradable photopolymers such as polyvinyl alcohol (PVA), polyethylene glycol (PEG), Poly (ethylene glycol) diacrylate (PEGDA), and so on.[7]

Publication trends of different 3D printing techniques for biomedical applications

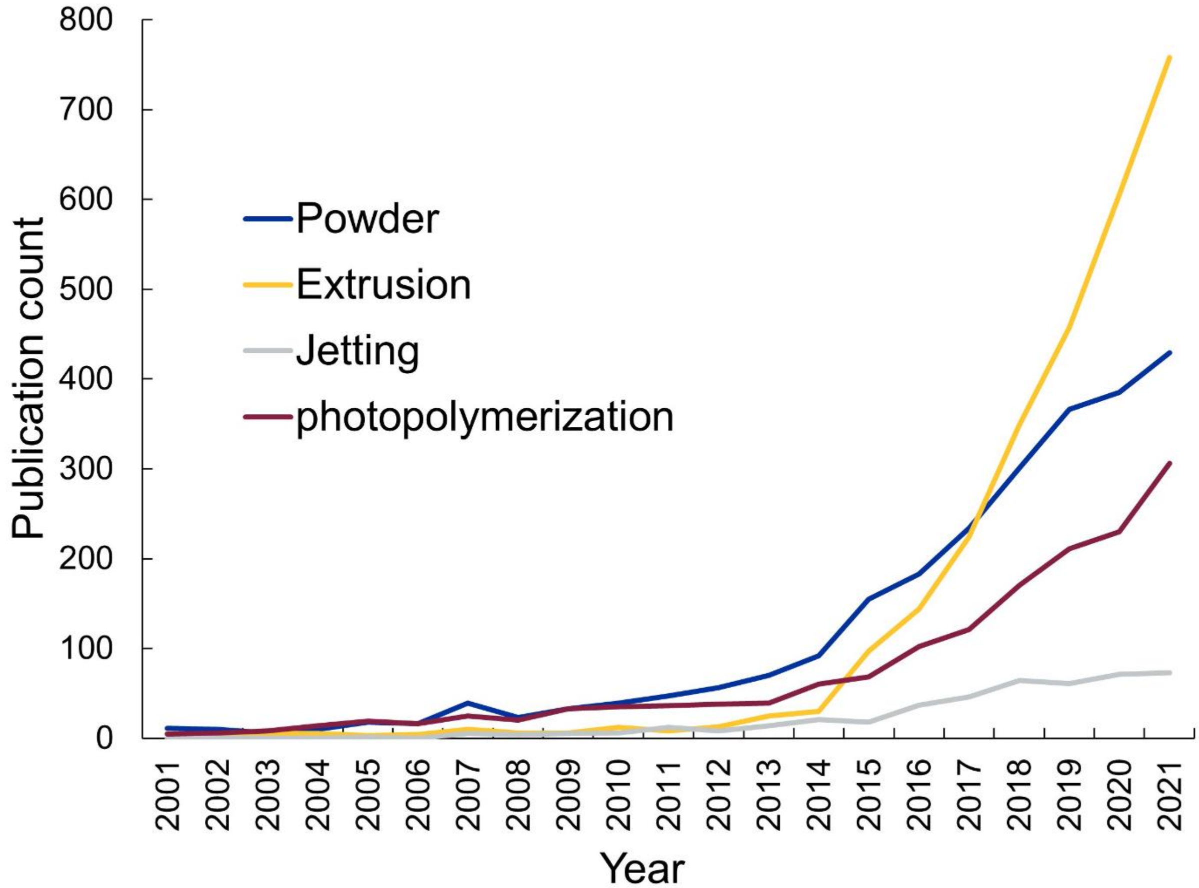
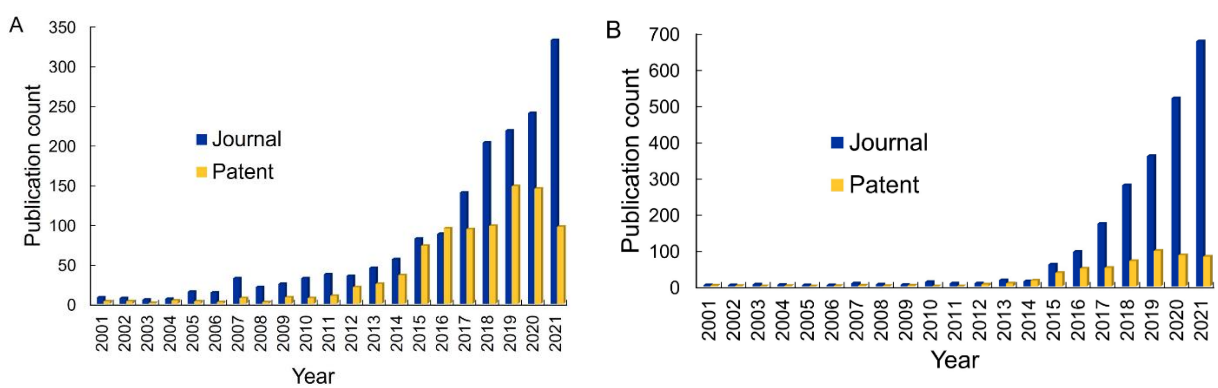


Figure 7. Publication trends for different techniques of 3D printing used in biomedical applications



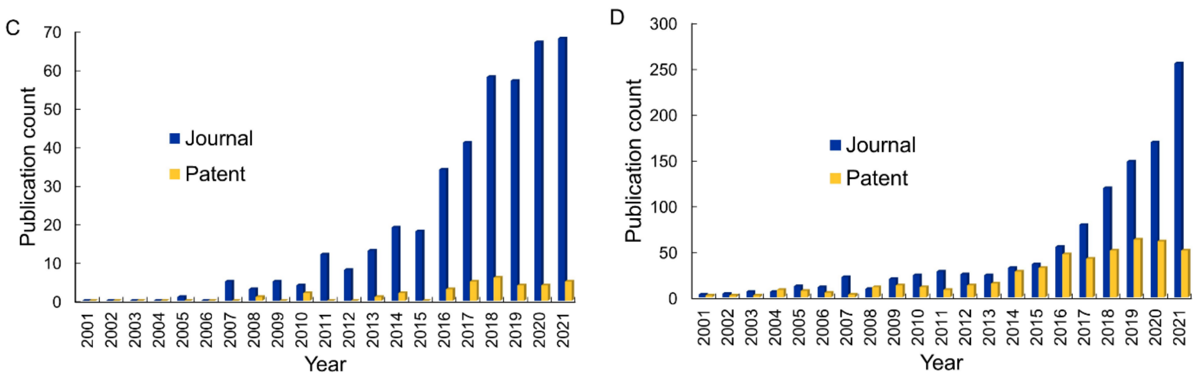


Figure 8. Comparison of journal and patent publication trends for 3D printing techniques used in biomedical applications. (a) powder; (b) extrusion; (c) jetting; (d) photopolymerization.

To look further into the publication trend based on four major 3D printing technologies—powder bed fusion, jetting, extrusion, and photopolymerization, the annual publication counts for the technologies from 2001 to 2021 are shown in Figure 7. Extrusion grows dramatically after 2014 and becomes the mainstream among these four methods in 2021 (Extrusion: 48.4%; Powder bed fusion: 27.4%; Photopolymerization: 19.5%; Jetting: 4.7%). As the timeline indicates, powder bed fusion and extrusion are two major techniques (34.6% and 37.9%, respectively).

The journal and patent publication trends for each technology are revealed in Figure 8. For each technique, journal publication volumes are higher than patent publications. This phenomenon matches the annual publication trend (Figure 1). Only 7.4% of publications in jetting are patents; most jetting-related publications are devoted to journal articles (92.6%). For other techniques, the patent publication counts are lower than the journal publications (as patents, extrusion: 18.5%; powder: 35.0%; photopolymerization: 30.4%), but the proportions of patents are higher than that of jetting. We can expect the growth of patent publication in the future upon the commercialization of new inventions in 3D printing technologies for biomedical applications.

Substances-Based Analysis in Biomedical Application

Herein we categorized substances into polymers, inorganic, and hydrogels. We selected several common substances used as 3D printing materials, and the introduction is also given in the following section.

Synthetic Polymers

Polycaprolactone (PCL) and poly(lactic acid) (PLA) are both linear polyesters with low melting points. PCL has been extensively utilized with 3D printing technologies for medical applications because of its low melting point (60 °C). PCL is applied to liver-on-a-chip, bone generation, cartilage reconstruction, etc.[8, 9] PLA, a biodegradable thermoplastic polymer, is also used for tissue engineering purposes as well as medical implants, screws, stiches.[10] Polyethylene is used for medical implants, even extended to facial and cranial reconstruction with its porous high-density polyethylene form .[11] Poly(vinylpyrrolidone) can perform as the 3D hierarchical porous structures for further tissue engineering.[12] Polystyrene is capable of being adapted to display bioreplication/biotemplating.[13] Poly(methyl methacrylate) is mainly applied to dental materials like orthodontics and drug loading/drug delivery.[14, 15, 16]

Synthetic Hydrogels

Hydrogels are commonly used materials that can absorb lots of water/fluid and swell in the water.[17] They feature crosslinked structures with biocompatibility for 3D printing-based medical applications. Polyethylene glycol (PEG) is a hydrophilic polymer that has low toxicity. PEG-based hydrogel has good biocompatibility and has been widely used in 3D printing applications as biomaterial ink to produce vessels and cartilages.[7] Its derivative, polyethylene glycol diacrylate (PEGDA), is also applicable to tissue engineering of vascular constructs .[18] Poly(vinyl alcohol) (PVA)[19] is a water soluble polymer that can form PVA-based hydrogel as a biomaterial for 3D printing applications such as vascular and cartilage tissue engineering. [7] Poly(lactic-co-glycolic acid) (PLGA) [20] is the co-polymer made from lactic acid and glycolic acid. PLGA has been used for bone and cartilage implants due to its low melting point (120 °C) and biocompatibility.

Natural Hydrogels

Alginate and hyaluronic acid can provide extracellular matrix-like environments for cellular movements due to their mechanical and biological properties that make them biocompatible to form alternative cartilage, vascular structures, skin, and muscle.[7] Chitosan is mainly used for cartilage, bones, and skin.[21, 22] Cellulose has been utilized with different types and derivatives such as nanocelluloses to form cartilage tissue. Cellulose usually combines with other known hydrogels and is printed as biomedical devices.[23, 24]

Top Inorganic Substances

Hydroxylapatite (HAp, $Ca_5(PO4)_3(OH)$), is heavily used as the filler in dental material and bone repair. [20] Tricalcium phosphate is also anther filler for bone regeneration/repairs in combination with other biopolymers. [20] Graphene oxide and graphene are both used in bone regeneration/repair [25, 26]as the additive to reinforce the polymers. Graphene filament combined with PLC is printed as the 3D electrode to become the biosensor.[27] Zirconium dioxide (ZrO_2) is used as a filler combined with other polymers in dental prosthesis or bone implant materials. [28, 29, 30] Alumina, silica, and silicon are usually utilized as the dental crown model, artificial teeth, and denture base materials.[31, 32, 33] Carbon nanostructures are utilized to form 3D printing implants for bone tissue remodeling[34], and carbon fibers can form microlattice 3D hybrid scaffold for tissue engineering.[35] Titanium/Ti_6Al_4V are involved in bone tissue remodeling, bone defect repair, knee implant, etc.[34, 36, 37] Gold nanotubes or nanowires can be combined with decellularized extracellular matrix to form hydrogel for tissue engineering[38] or directly used for medical implant[39].

Publication trends for substances and concepts used in biomedical 3D printing studies

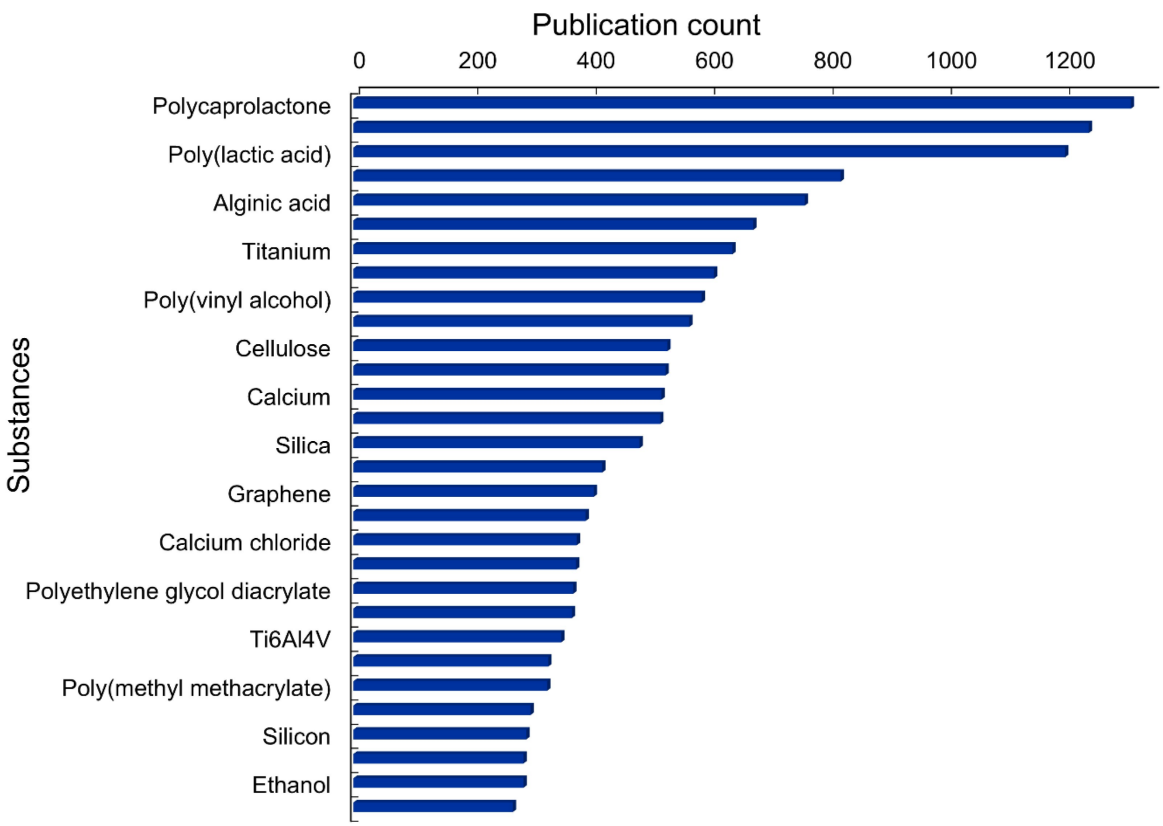


Figure 9. Top 15 substances that appeared most frequently in publications on biomedical applications of 3D printing

We extracted publication counts based on substances related to 3D printing in medical applications, as shown in Figure 9. Polycaprolactone, poly(lactic acid), and alginic acid are the top three commonly used 3D printing biomaterials for medical implants, bone/cartilage repair, and dental materials.

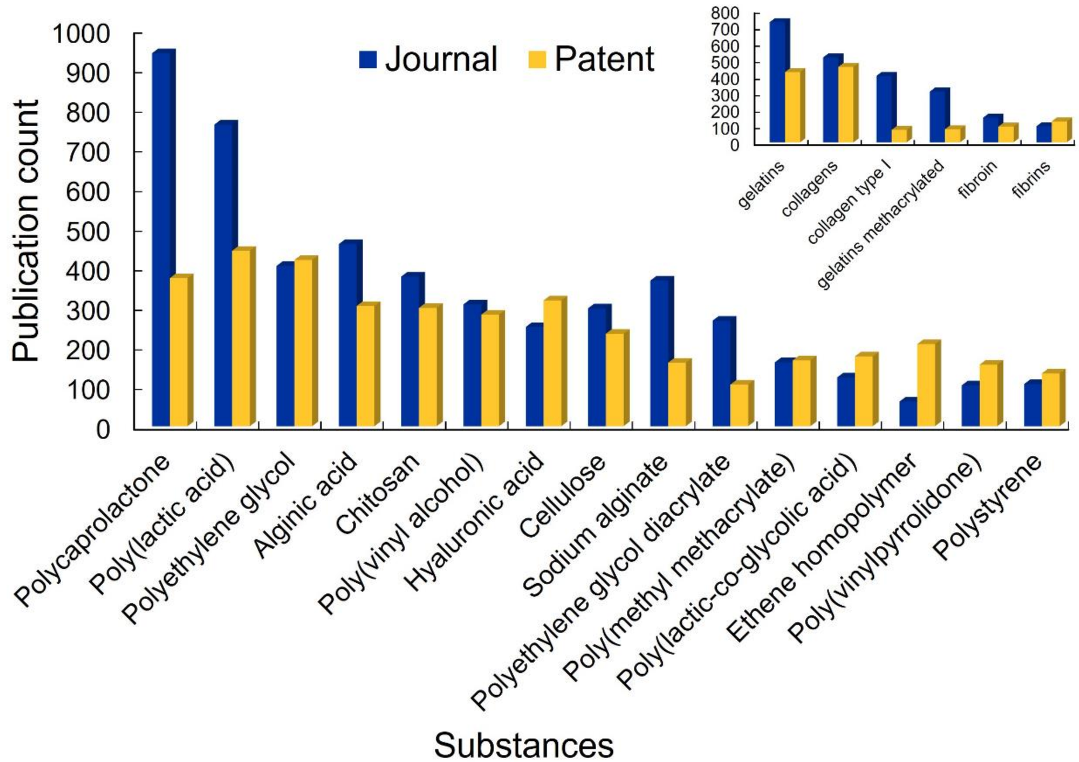


Figure 10. Journal and patent publication volumes involving top 15 frequently used **polymers** (Inset graph represents the number of publications involving unspecified substances) in publications on biomedical applications of 3D printing

Next, the journal and patent publication volumes for top substances for different subfields are studied. Under the polymer category (Figure 10), polycaprolactone, poly(lactic acid), and polyethylene glycol are the first three polymers with the higher number of publications. Majority of these polymers are used in tissue engineering cartilage and bone, or in constructing medical implants. We also searched the unspecified substances, “gelatins, collagens, fibroin, fibrins and their derivatives” in our database. Gelatins and collagens are the two major natural polymers applied as hydrogels in various fields across different 3D printing techniques.

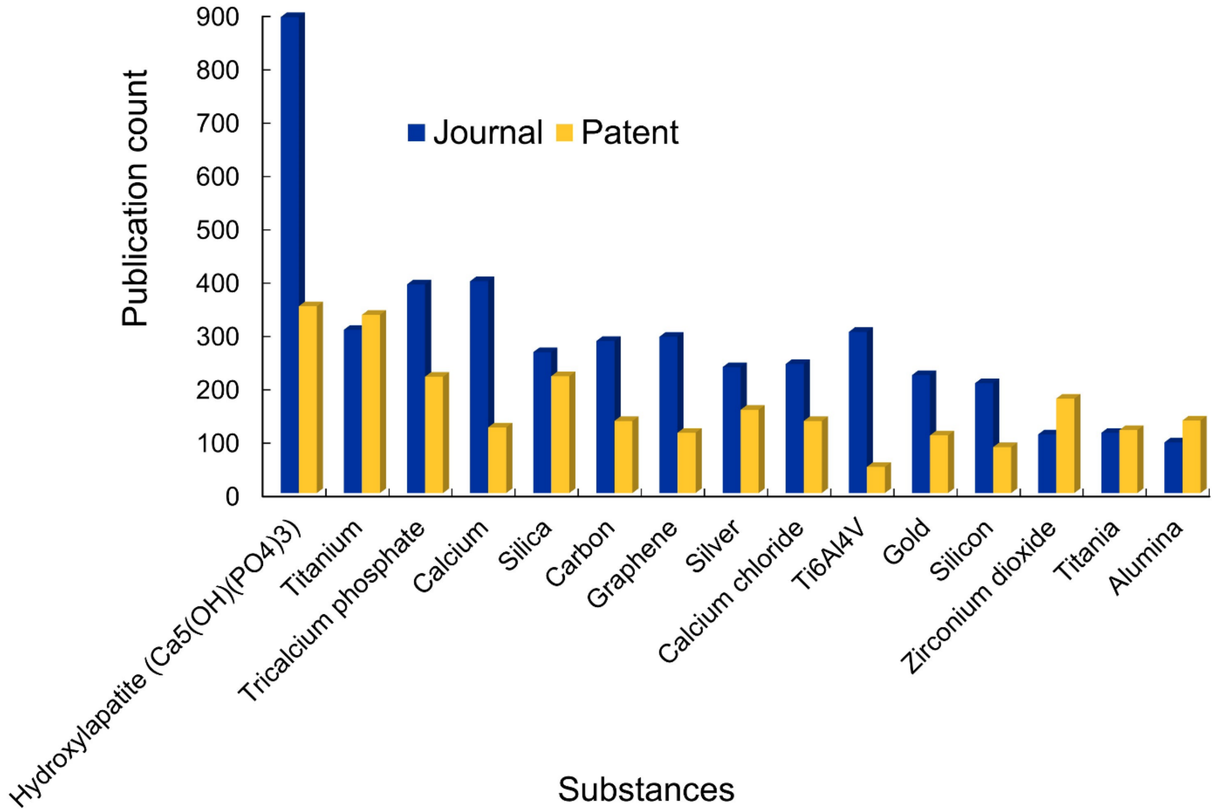


Figure 11. Journal and patent publication volumes involving top 15 frequently used **inorganic substances** of biomedical applications of 3D printing

In Figure 11, hydroxylapatite (HAp, $Ca_5(OH)(PO_4)_3$)-related publications are the highest among the top 15 inorganic substances for dental materials and bone repair. Tricalcium phosphate (TCP) is another major inorganic material with myriads of inventions in bone repair/regeneration. HAp and TCP are both capable of forming hybrid polymers with other natural/synthetic polymers for bone tissue engineering. This enhanced their development in journals over patent publications. Titanium/Ti_6Al_4V are mainly featured as the materials for bone implants/repairs using 3D printing techniques. The journal/patent publication differences are trivial for titanium materials but Ti_6Al_4V. For Ti_6Al_4V alloy, we are expecting more patents to be granted in the foreseeable future.

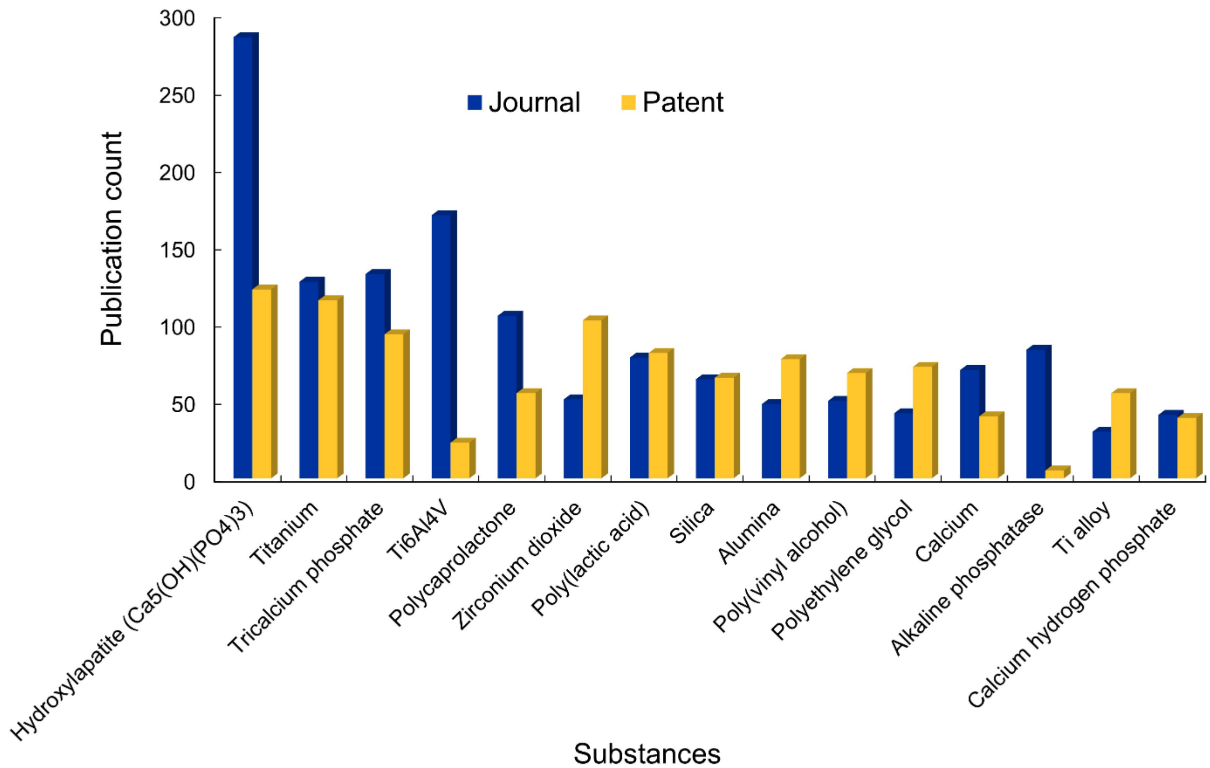


Figure 12. Journal and patent publication volumes involving top 15 frequently used substances in biomedical applications of **powder**-based 3D printing

Figure 12 shows the most used substances in powder-based 3D printing studies. Inorganic substances are more commonly used. Of these inorganic substances, HAp, TCP, titanium, and Ti_6Al_4V are the most popular. The most used polymers are polycaprolactone and poly(lactic acid).

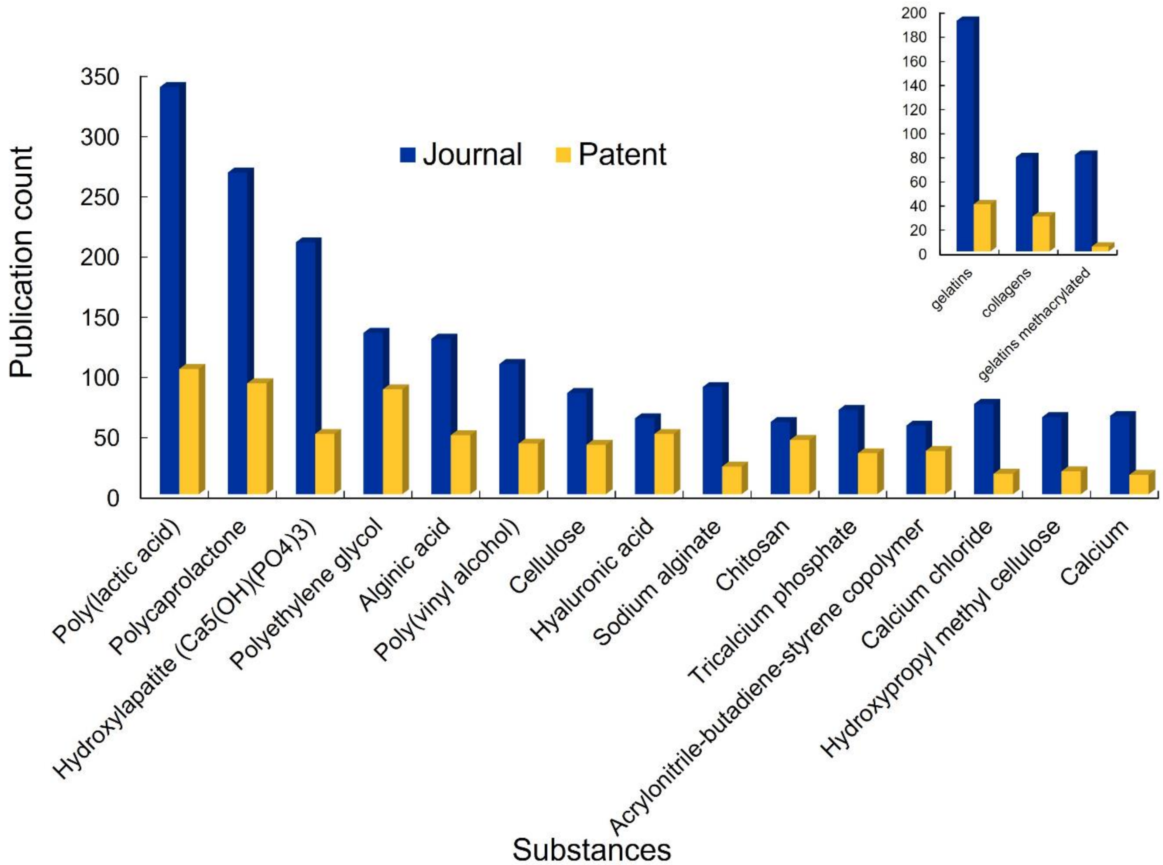


Figure 13. Journal and patent publication volumes involving top 15 frequently used substances (Inset graph represents the number of publications involving unspecified substances) in biomedical applications of **extrusion**-based 3D printing

For the extrusion-based technique (Figure 13), synthetic polymers like poly(lactic acid) and polycaprolactone are the major substances involved in this technique, as well as natural polymers like alginic acid and its sodium salts. Gelatins and collagens (which exist in CAS database as unspecified substances), are also widely used in extrusion-based 3D printing.

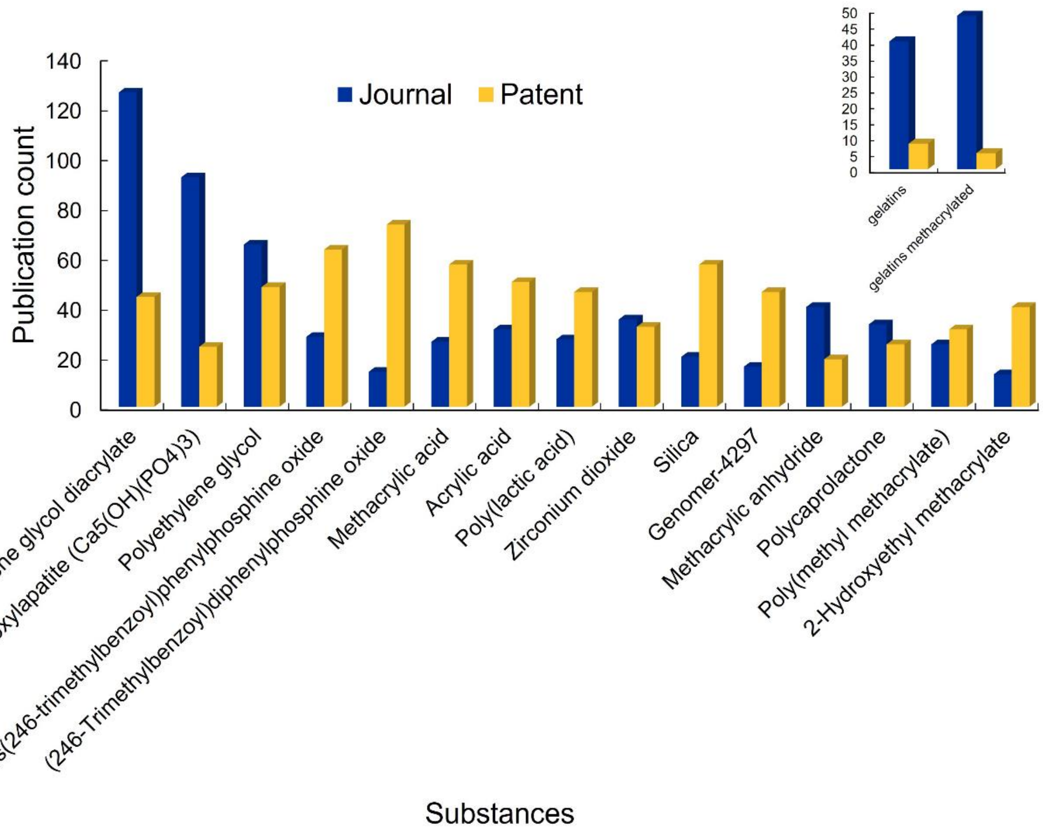


Figure 14. Journal and patent publication volumes involving top 15 frequently used substances (Inset graph represents the number of publications involving unspecified substances) in biomedical applications of **photopolymerization**-based 3D printing

Polyethylene glycol diacrylate (PEGDA) and HAp powders form composites via photopolymerization 3D printing and are the top two substances occurring in photopolymerization-related documents (Figure 14). Acrylic monomers like methacrylic acid and acrylic acid, as well as photoinitiators, for instance, bis(2,4,6-trimethylbenzoyl)phenylphosphine oxide, led to high patent numbers because they are commonly used as the starting materials for synthetic hydrogels.

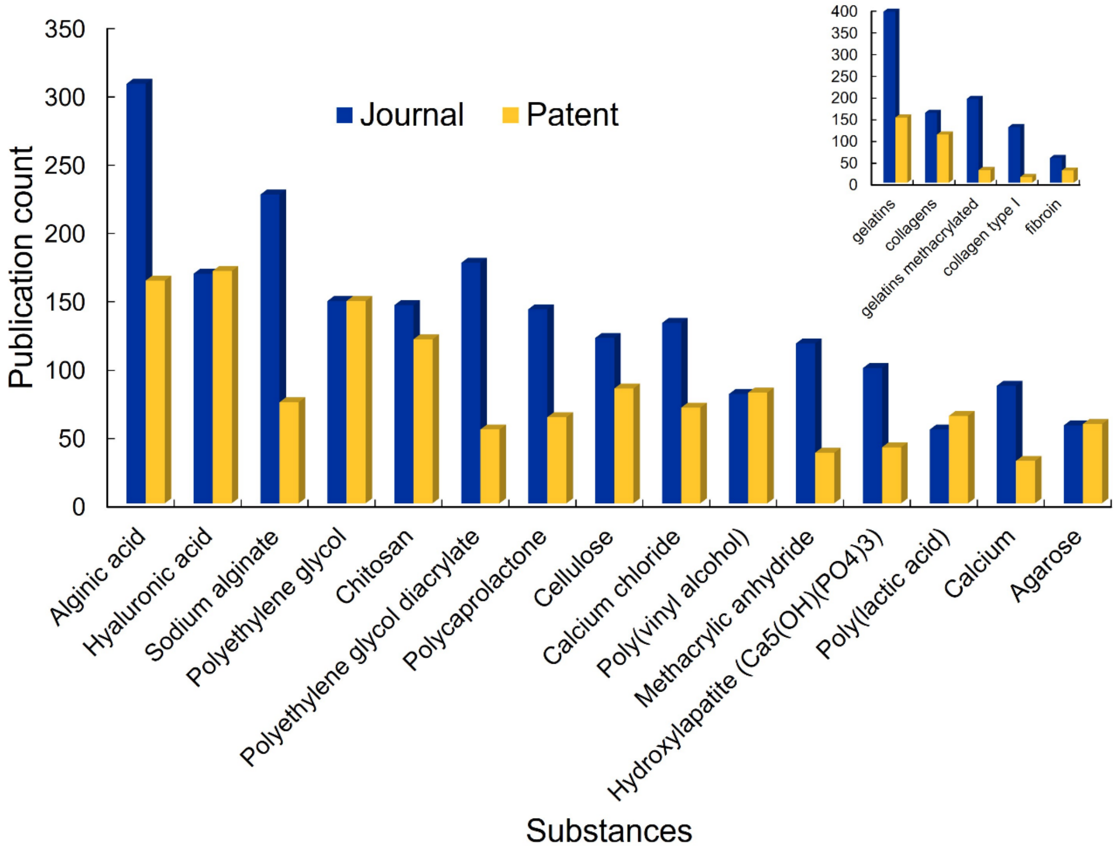


Figure 15. Journal and patent publication volumes involving top 15 frequently used substances (Inset graph represents the number of publications involving unspecified substances) for biomedical 3D printing involving **hydrogels**

Publication volumes for top substances occurring in hydrogel-related documents are shown in Figure 15. Alginic acid, hyaluronic acid, sodium alginate, and chitosan are the major natural hydrogels used as feeding materials for 3D printing. Polyethylene glycol and polyethylene glycol diacrylate are the two major synthetic hydrogels. Gelatins and collagens are the two unspecified substances that contributed the most as hydrogels to biomedical 3D printing. This trend is similar to those in Figure 24. This is not surprising considering that hydrogels are the most widely used materials for 3D bioprinting.

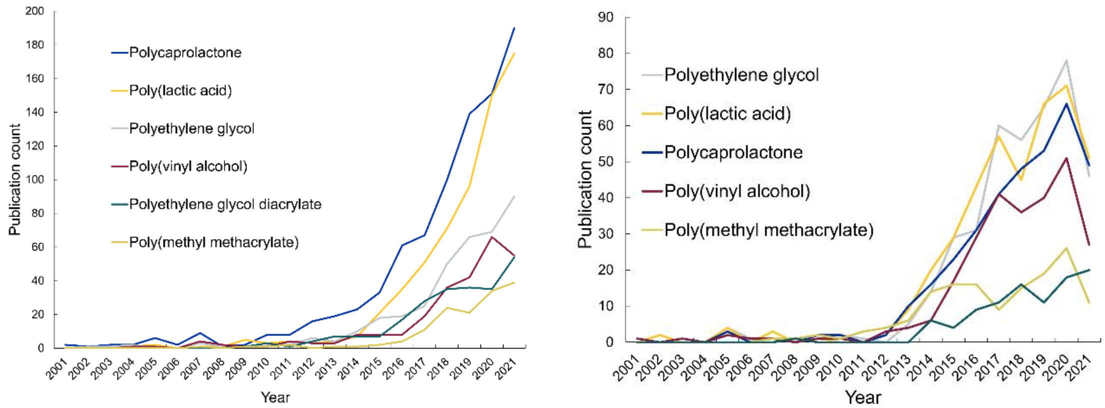


Figure 16. Annual journal (left) and patent (right) publication volumes for top **synthetic polymers** used as 3D printing materials in biomedical applications

The annual trends of publications involving several top synthetic polymers, natural polymers, and inorganic substances are shown in Figures 16-18, respectively. Polycaprolactone and poly(lactic acid) are two leading synthetic polymers used for biomedical applications of 3D printing in journal publications. In patent applications, poly(lactic acid), polyethylene glycol, and polycaprolactone feature similar invention counts throughout the years. Interestingly, all synthetic polymers have patent publication declines in 2021. This may be due to the delay of the issuance of patents.

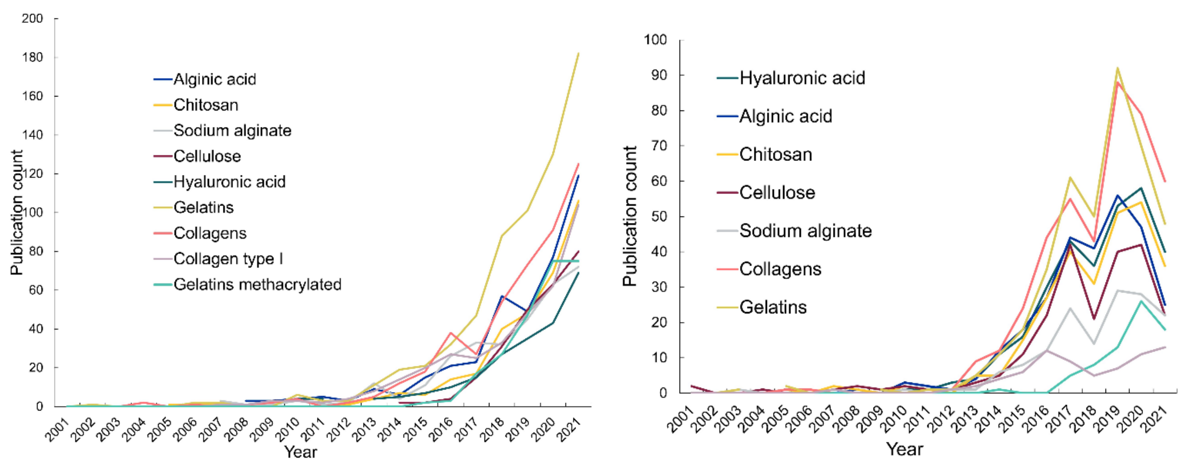


Figure 17. Annual journal (left) and patent (right) publication volumes for top **natural polymers** used as 3D printing materials in biomedical applications

Figure 17 shows that gelatins, collagens, and their derivatives result in significant numbers of publications in both journals and patents. Alginic acid is focused by people for journal publications, and hyaluronic acid is reported by more researchers as patents instead. In 2021, the number of patent publications in several polymers decreased. This may have resulted from the progress of patent issuing in 2021.

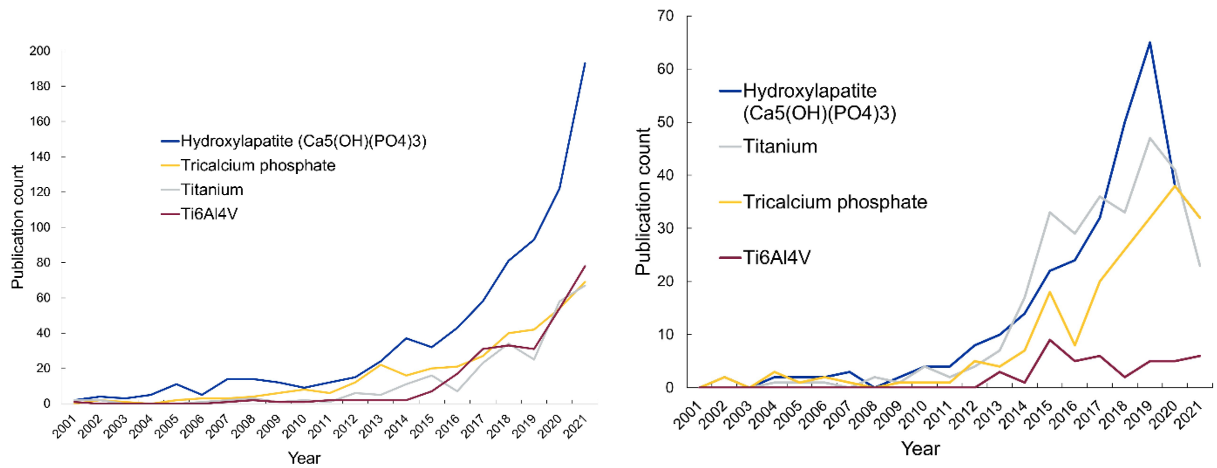


Figure 18. Annual journal (left) and patent (right) publication volumes for top **inorganic substances** used as 3D printing materials in biomedical applications

Figure 18 indicates that HAp is leading the inorganic substance as 3D printing material in biomedical applications. The trend between journals and patents (the number of journals is higher than patents) displays that HAp has been investigated and has the potential to be commercialized in the foreseeable future. Titanium, TCP, and Ti_6Al_4V also have steady development, but Ti_6Al_4V still has relatively lower patent publication counts than other substances. We can look forward to seeing the rapid patent growth in Ti_6Al_4V as a 3D printing material. The decline of patent publication count in 2021 may be related to the progress of patent issuing.

3D Printing in Biomedical Applications

3D printing techniques have been extensively applied to various fields. Herein, we focus on the recent biomedical applications using 3D printing. 3D printing in the biomedical field can include biofabrication via bioprinting or 3D printing non-biological materials used for medical purposes. Biofabrication is an automated process that generates a product with biological functions from biomaterials, living cells, or other bioactive molecules or constructs using bioprinting or bioassembly, followed by tissue maturation.[40]

Tissue and Organ Fabrication

Tissue and organ fabrication is enabled through tissue engineering principles, utilizing cells, biomaterials, and technology to produce constructs that mimic the function and design of its counterpart within the human body.[41] Bioprinting, a form of extrusion printing, is used in tissue engineering to create these constructs. The most common is to produce a scaffold to load cells. The scaffold can be made of naturally derived polymers, i.e. alginate, chitosan, gelatin, or collagen, or synthetic polymers, polycaprolactone (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA).[41] The cells attach to these scaffolds and proliferate, developing their own extracellular matrix. From here, the cells can mature and are regulated with the appropriate stimuli.

Cartilage Fabrication

Tissue engineering applications with cartilage have become a popular focus in the biomedical field. Articular cartilage and menisci can be fabricated with bioprinted scaffolds loaded with stem cells.

This technique allows for the development of complex structures, the different types of cartilage, and it can be constructed based on the patient's specific needs.[42] The use of stem cells in cartilage fabrication, along with other tissues such as bone, prevents immune response rejection and its paracrine activity. In recent years, the incidence of bone disorders and conditions has increased with limitations and complications. To combat the complications of bone grafting, biomaterials in bone-tissue engineering have expanded over the years. The objective is to prepare materials that are introduced to the bone defect or injury and then remodeled by the patient's own cells. For instance, the hydrogel-like freeform reversible embedding of suspended hydrogels (FRESH) can be utilized as the 3D printing materials to retain the initial shape for further modification. The mechanical weakness of hydrogels can be improved with nanomaterials, such as graphene, to enhance its properties. Beyond natural polymers, synthetic polymers, bioceramics, biodegradable metals, and carbon-based nanomaterials are commonly used.[43]

Muscle Tissue Engineering

Muscle tissue engineering has also garnered attention over the years (**Figure 21**). Gelatin methacryloyl (GelMA) is a bioink that can mimic the native skeletal muscle tissue environment.[44] This hydrogel has promising potential as it is mixed with alginate for desired bioprinting viscosity. Cell-laden GelMA microfibers are bundled. By working on the swelling properties and folding abilities, the cell-laden GelMA microfibers can be used to mimic the structure of different skeletal muscle tissues.[45] For smooth muscle tissue fabrication, collagen laden with smooth muscle cells form line patterns with layer-by-layer deposition and demonstrate uniform cell seeding with controlled resolution.[46] Cardiac muscle tissue or heart organ applications commonly use hydrogel bioinks. Rather than creating an entire organ, cardiac patches can be created from the patient's fatty tissue.[47] The cells from the patient are reprogrammed to pluripotent stem cells, and the collected extracellular matrix is processed into a hydrogel. This eliminates the risk of implant rejection and is a biocompatible scaffold. The result is a bioprinted thick, vascularized, and perfusable cardiac patch that will match the immunological, biochemical, and anatomical properties of the patient.[47] The patch allows stem cell adhesion, differentiation, and proliferation to a damaged heart, improving wound healing and functional preservation.[48]

Skin Tissue Fabrication

Skin tissue fabrication has posed unique challenges in tissue engineering. Engineered tissue cells can be used to restore or replace damaged tissues and organs. Cell placement in the tissue construct is limited and accounts for the spatial relations between the extracellular matrix and cells. In general, a hydrogel scaffold is used, and the cells are integrated with a thermal inkjet printer to deposit the bioink. To mimic the complexity of the skin tissue structure that allows different cells to proliferate, a laser assisted bioprinting (LaBP) technique is used for the positioning of different cell types in an exact 3D spatial pattern.[49] Tissue engineered skin substitutes have widespread applications, from aiding patients in tissue regeneration to replacing animals in research, whether for pharmaceutical or cosmetic needs. Utilizing methods with a cellularized skin equivalent can revolutionize the field.

Pharmaceuticals

Manufacturing pharmaceuticals via 3D printing has revolutionized how we develop drug products, implants, drug delivery systems, etc. The field of pharmaceuticals faces shortcomings when it comes to personalized medication, and additive printing has provided potential solutions. There are several techniques that can be used to print drug products: Powder-based printing, extrusion-based printing (fused deposition modeling (FDM), pressure-assisted microsyringes (PAM)), stereolithographic printing (SLA), selective laser sintering (SLS), inkjet printing, digital light processing (DLP), etc. Extrusion-based printing has gained traction due to its low cost, compatibility with a variety of feeding materials to adjust the geometry and polymer for drug delivery purposes, and the ability to print at room temperature using PAM.

There are two types of extrusion-based 3D printing. FDM is the most common as it is cost-efficient and uses thermoplastics. Thermoplastic polymers such as polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), and poly(lactic acid) (PLA) are used as a drug carrier and require thermo-resistant drug molecules. PAM requires lower temperatures than FDM to print products, allowing for desired viscosity during drug formulation. A paste or gel that is shear-thinning is often ideal as it retains shape and structure after going through the printer nozzle. PAM does require a drying time unlike FDM, during which the product can deform or shrink.

3D printing offers techniques that make personalized medication viable. Current methods of production feature limitations on drug release rates, and personalized medication pertains to manually splitting tablets or measuring out liquid dosages. 3D printing enables a constant release rate as it can fabricate different polymer matrices and provide more precise methods of measurement. The active ingredient dose is controlled by changing the printed volume using computer software. More complex shapes can also be created, such as the torus shape to an increased surface area. The polymers used carry a large portion of the ingredients and serve different purposes whether it be to bind, disintegrant, compress, dilute, or act as a filler. These include and are not limited to: Carbopol, Ethylcellulose (EC), Eudragit, Hydroxypropyl Cellulose (HPC), Hydroxypropyl Methylcellulose (HPMC), Polycaprolactone (PCL), Polylactic Acid (PLA), Polyvinyl Alcohol (PVA), Polyvinylpyrrolidone (PVP), Poly(Ethylene Glycol) (PEG), and Soluplus.[50]

Orthopedics and Prosthetics

3D printing has opened new capabilities in creating prosthetics and orthopedics. Fused Deposition Modeling, FDM, is the most common printing method due to its efficiency and lower costs. Thermal Inkjet Printing and Selective Laser Sintering are two other methods seen in prosthetic fabrication. X-rays, MRI, and CT scans can be translated into digital .STL 3D print files, offering customized designs and fits. Acrylonitrile butadiene styrene (ABS) and poly(lactic acid) (PLA) are the most common materials used in prosthetics. Prosthetics made from flexible materials use NinjaFlex, a thermoplastic polyurethane, or Filaflex, a thermoplastic elastomer. SLS printers will use a nylon-based material, and Polyjet printers will use a photopolymer resin. However, with photopolymers, there is an issue with the material degrading in sunlight.[51]

Flexible materials have provided more options with body parts and capabilities. With the progression of prosthetics, prosthetic ears are developed with the ability to detect electromagnetic frequencies. These prosthetics are fabricated from silicon, chondrocytes, and silver nanoparticles. 99% of hearing aids are also 3D printed as it offers customization based on the patient's size, location, etc.[52]

Orthopedic implants are also projected to expand. 3D printed implants are especially prevalent in orthopedic oncology. Malignant bone tumors can affect any part of the skeleton, and bone tumors have bimodal incidence, affecting young patients especially. Limb salvage surgery is a common surgery for malignant bone tumors and is individualized. 3D printed metal implants are used to reconstruct massive bone defects after a wide excision is made. However, if the bone tumor is near the joint or physis, segmental reconstruction is implemented. Modular implants are not utilized when reconstructing unusual sites, like the pelvis. Titanium alloy powder is used for bone reconstruction, and common printer types include electron beam melting or selective laser melting. Implants can not only focus on filling bone defects but attempt functional reconstruction.[53]

Additional 3D printed implants on the skeleton include 3D printed polyetherketoneketone (PEKK) skull implants where no shaving is necessary as the implant is a customized design and fit.[52] There are also maxillofacial, spinal, and dental implants. Dentistry often uses titanium or ceramics for dental implants. Bioceramics offers an opportunity to produce cell-seeded implants that are patient-specific.[54] 3D printing shows promise as it is personalized to the anatomy, color, shape, and size of the patient without the need to adjust the production machine.

Others (Surgical Instrumentation, Microfluidics, Anatomical Models)

3D printing can also be applicable in other instances in the biomedical field. Microfluidic chips provide a cheaper, more resource-effective potential for drug discovery and tissue engineering. Lab-on-a-chip is a microfluidic device that can be used to detect analytes and biomarkers. Organ-on-a-chip is a similar concept, utilizing these chips to mimic a specific functionality of an organ to test in lab. Microfluidic chips can be created by different 3D printing techniques, including stereolithography, multi-jet modeling, and fused deposition modeling.[55] Polymers such as hydrogels and other inorganic materials are used to make the chip design. Resins can be used to create microfluidic chips by stereolithography to detect Salmonella bacteria.[56] Polydimethylsiloxane (PDMS) is commonly used in microfluidic devices, especially with organ-on-a-chip. PDMS is transparent, flexible, permeable to oxygen, biocompatible, and relatively low cost. However, PDMS is hydrophobic and fluorescent to a degree, so surface modifications have been developed to improve PDMS chips. Other common materials for chips include glass and thermoplastics.[57] The variety of techniques and materials in 3D printing provide a broad spectrum of options.[56]

Anatomical modeling provides important tools for teaching and learning in medicine. Patient-specific models, or biomodels, can be 3D printed from the patient's MRI/CT scan data. This application shows the potential pathologies, accurate sizing and placement for future implants, accounts for unexpected anatomy, models surgical resection and reconstruction, and decreases overall surgery time with better planning.[58] There are three primary 3D printing methods for anatomical models: fused deposition modeling (FDM), powder bed fusion (PBF), and stereolithography laser curing (SLC). FDM is the most common due to its low cost and compatibility with thermoplastics. FDM-produced models' mechanical properties are useful for manipulation during surgical rehearsal. PBF works with high precision parts in materials like Nylon, a material ideal for sterilization. SLC also creates precise resin-based models. Coloring of the models also provides insight as areas of interest can be highlighted to stand out from the model and provide better understanding. Biomodels help not only medical personnel but also the patient by augmenting the patient's understanding and capacity for consent with better preoperative understanding.[58]

Publication trends for 3D Printing in Biomedical Applications

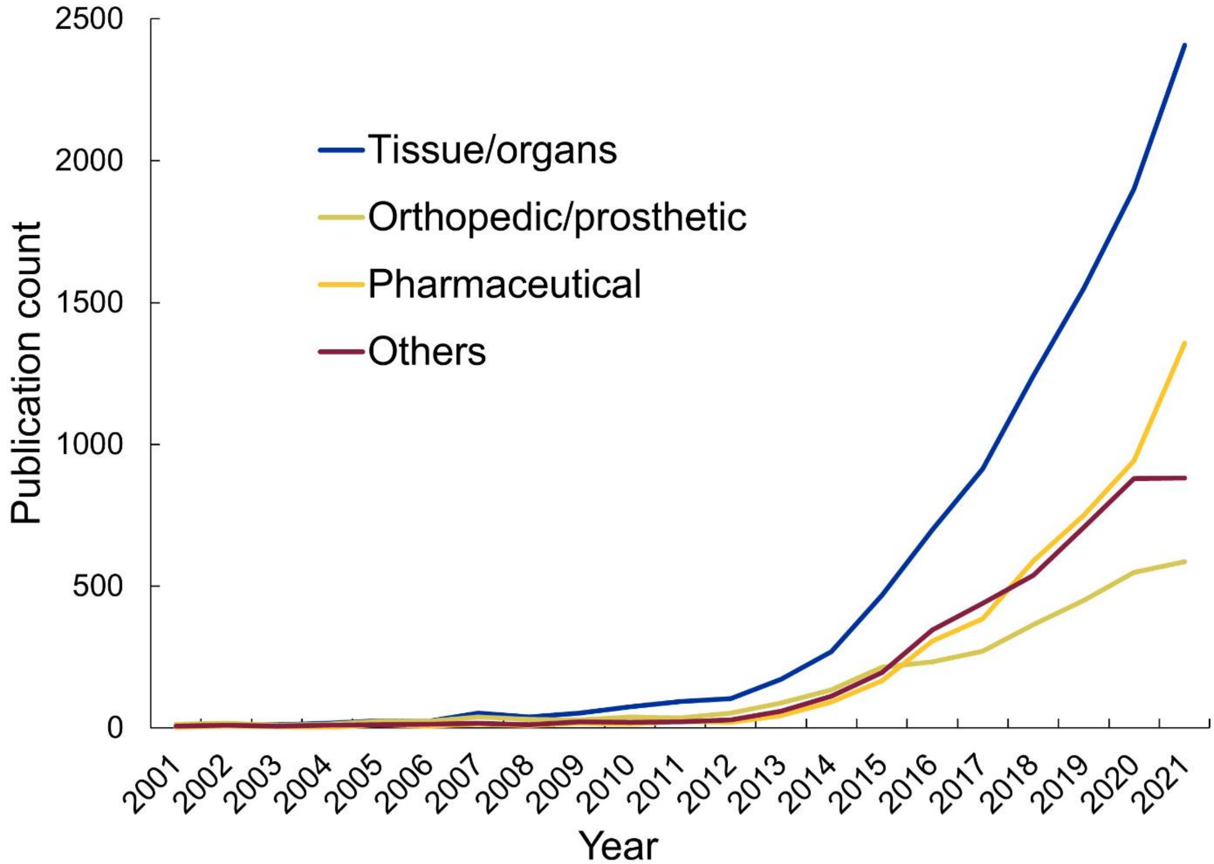


Figure 19. Annual publication trends for different biomedical applications of 3D printing

Next, we look at the trends of publications related to different biomedical applications of 3D printing. The publications of tissue/organs, orthopedic/prosthetic, pharmaceutical, and others were examined from our database (Figure 19). The analysis displays that the 3D printing technique has mainly been applied to tissue/organs-related fields since 2014. In 2021, 3D printing contributed to tissue/organs by 46.0%, followed by pharmaceutical (25.9%), others (16.8%), and orthopedic/prosthetic (11.2%).

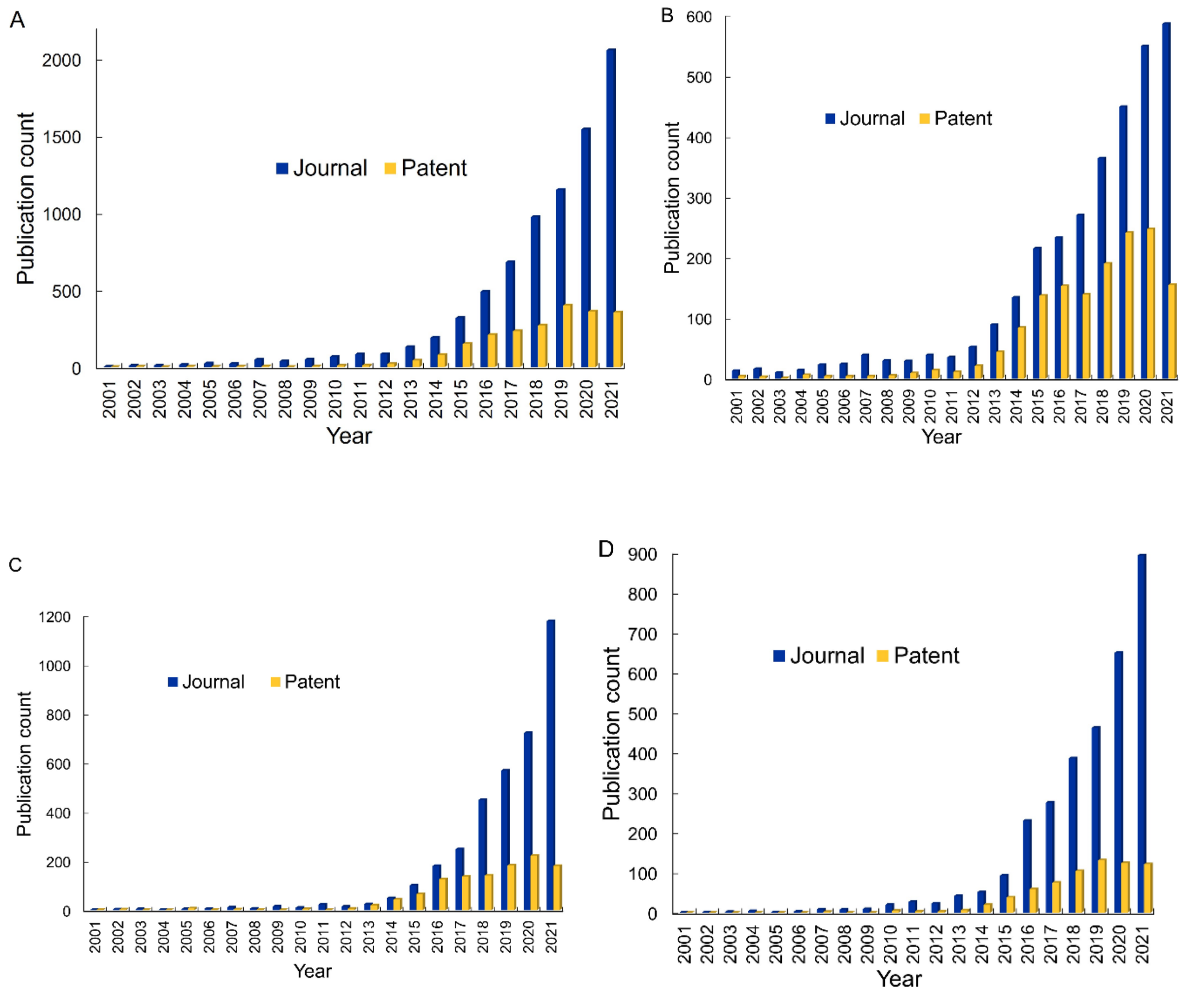


Figure 20. Comparison of journal and patent publication trends for different biomedical applications of 3D printing: (a) tissue/organs; (b) orthopedic/prosthetic; (c) pharmaceutical; (d) bioprinting.

Figure 20 provides more details for each application. Journal publication counts are considerably higher than the patent. This may indicate that the commercialization of inventions in journals and that the patent issuing process is usually time-consuming. We further analyzed a sub-field of tissue/organs—bioprinting. Figure 20D shows the total publication counts in 2021 are 1014, around 42% of the tissue/organs publication. Bioprinting has become one of the most attractive fields in tissue/organs application of 3D printing techniques.

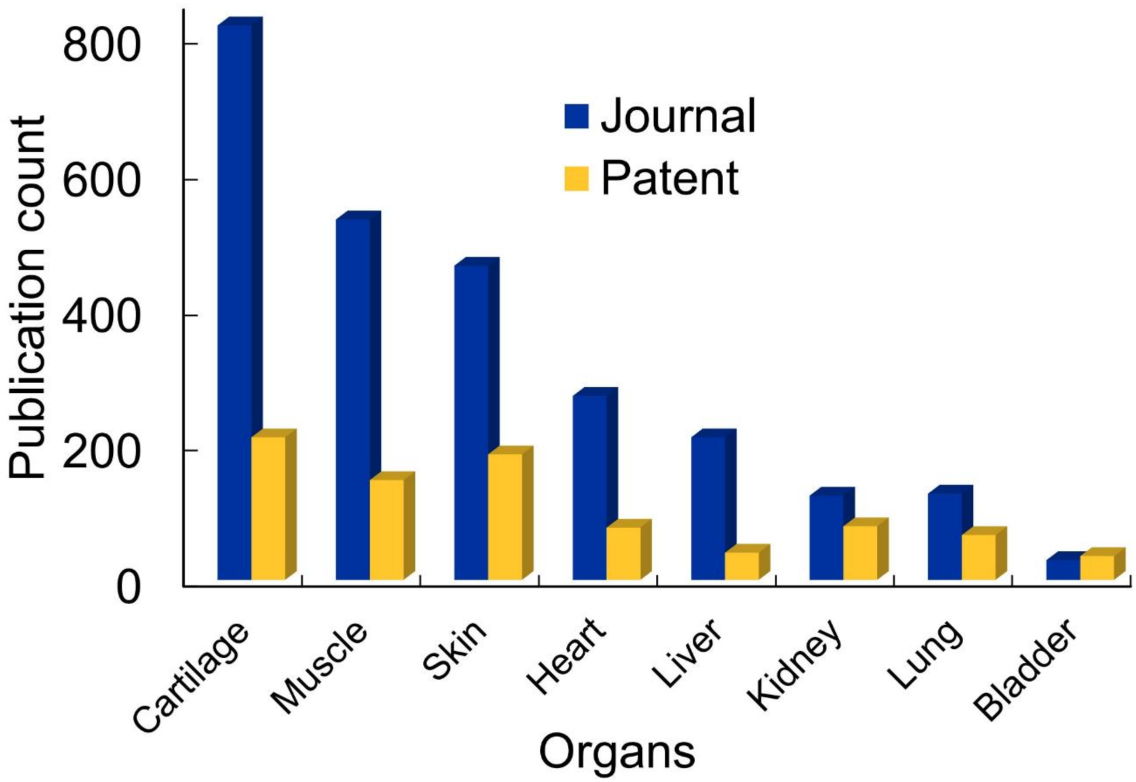


Figure 21. Journal and patent publication volumes involving application of 3D printing in **different organs** in the field of tissue/organs applications.

The publication counts related to different organs within the tissue/organs subfield are shown in Figure 21. Cartilage, muscle, and skin are the top 3 organs with the most journal publication counts, at least one time more than the corresponding patents. The total publication counts of selected organs in Figure 10 are 3411, approximately 16% of the documents selected from our CAS database. The development of tissue/organs application using 3D printing techniques is remarkable.

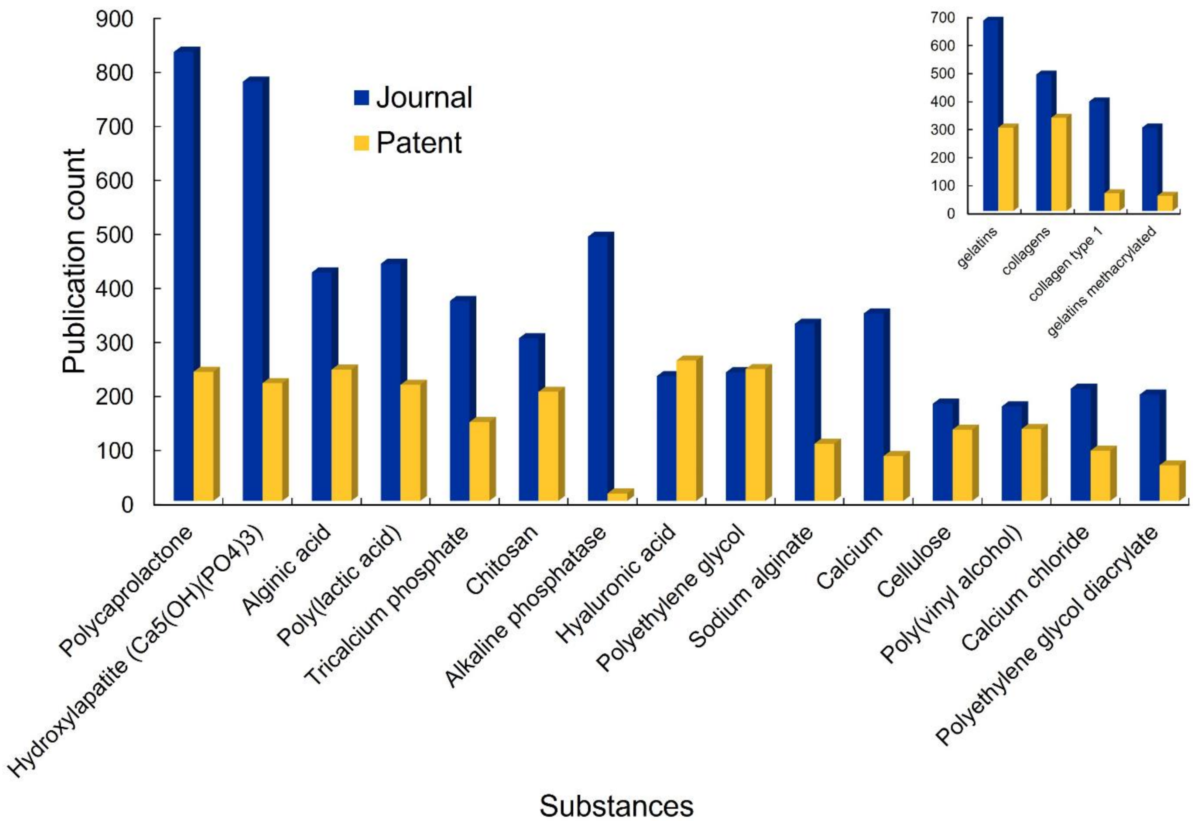


Figure 22. Journal and patent publication volumes involving top 15 frequently used substances (Inset graph represents the number of publications involving unspecified substances) for 3D printing applications in **tissue/organs**

The top substances occurring in documents related to tissue/organs and orthopedic/prosthetic applications are shown in Figures 22 and 23, respectively. 3D printing applications in tissue/organs mostly use natural polymers (in hydrogel form) like gelatin, collagen, alginic acid, and chitosan, while synthetic polymers like polycaprolactone and poly(lactic acid) are also used. HAp, TCP, and alkaline phosphatase are also applied to the tissue/organs. However, in this application, journal publication counts are considerably higher than the patents. This may represent the potential for development of the commercialized application using these substances.

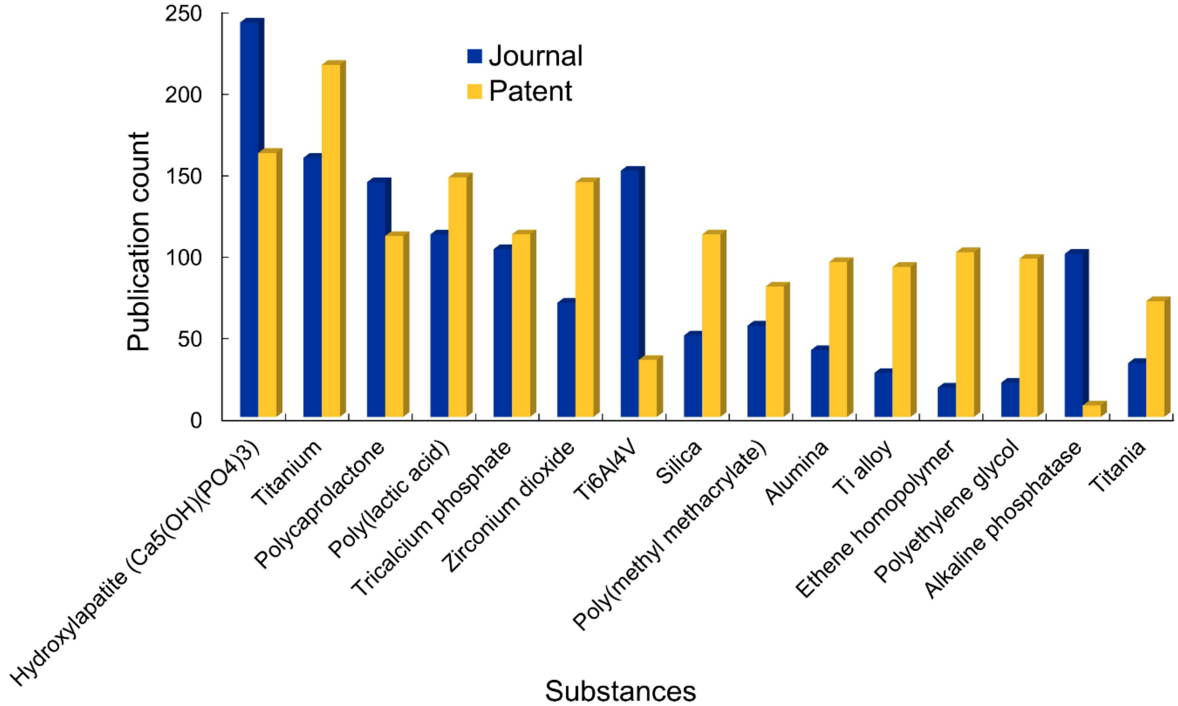


Figure 23. Journal and patent publication volumes involving top 15 most frequent substances for 3D printing applications in **orthopedic/prosthetic**

HAp, titanium, polycaprolactone, poly(lactic acid), TCP, zirconium dioxide, and Ti_6Al_4V contribute primarily to the orthopedic/prosthetic application. Silica, alumina, titania, and zirconium dioxide are commonly utilized as fillers in this field, and corresponding major publications are patents.

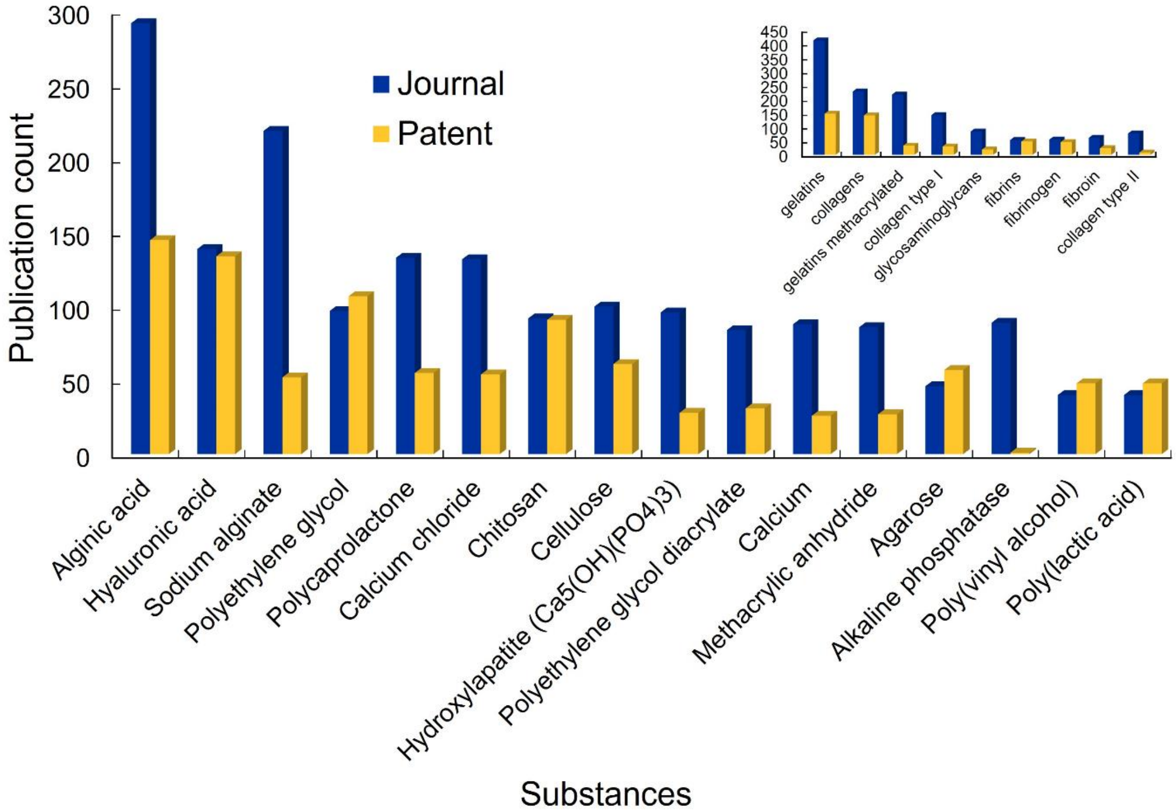


Figure 24. Journal and patent publication volumes involving top 15 frequently used substances (Inset graph represents the number of publications involving unspecified substances) for 3D **bioprinting**

Figure 24 shows the top substances occurring in documents concerning bioprinting. In general, natural polymers like alginic acid, sodium alginate, gelatins, and collagens as well as synthetic polymers like polycaprolactone, polyethylene glycol diacrylate, and methacrylic anhydride primarily resulted in journal publications. Alginic acid and sodium alginate have been extensively utilized as materials for 3D bioprinting applications. HAp also got involved in this area as an inorganic filler with other natural or synthetic polymers to form modified biopolymers for bioprinting.

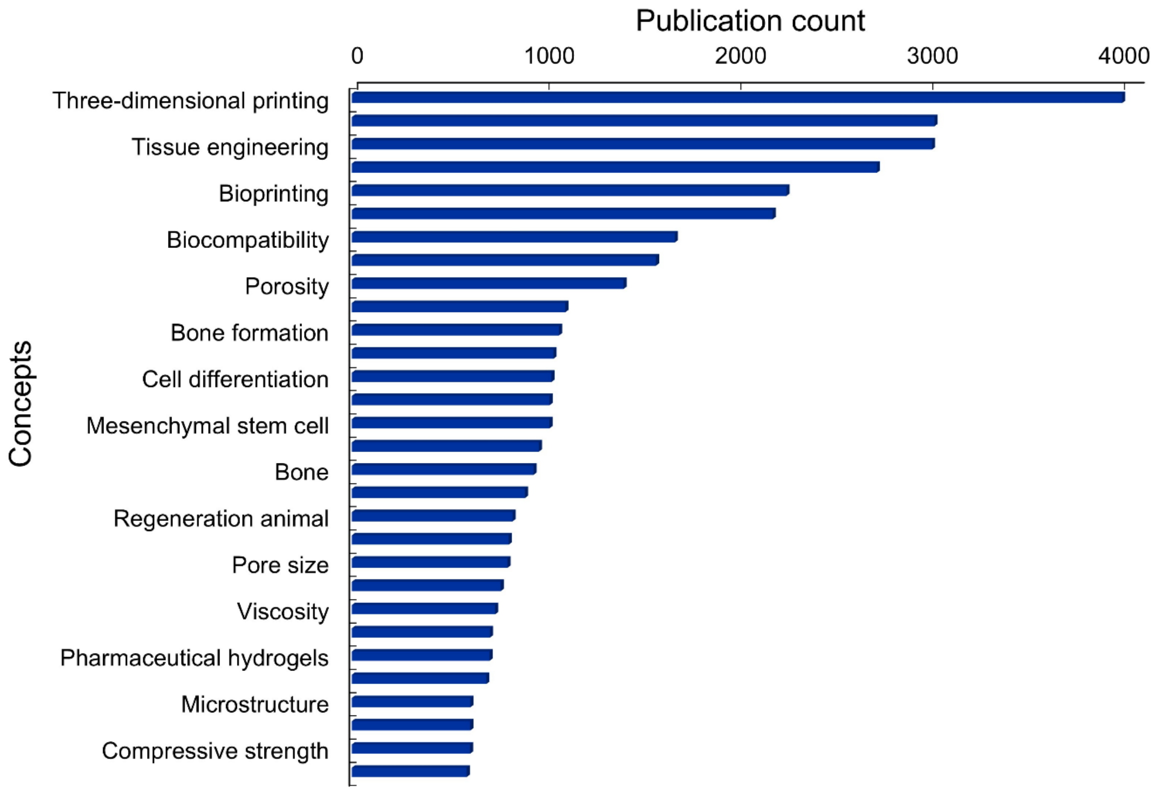


Figure 25. Top 15 concepts having appeared most frequently in publications on application of 3D printing in **tissue/organs**

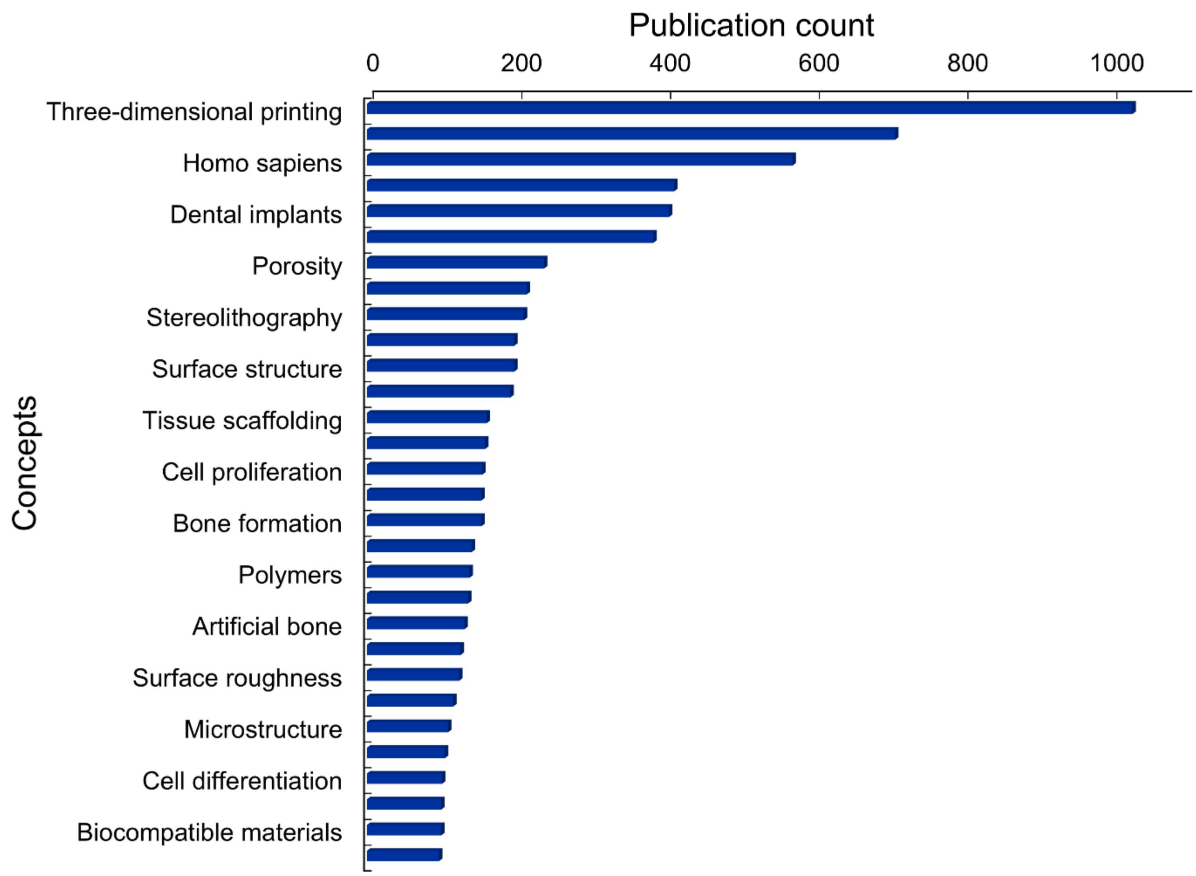


Figure 26. Top 15 concepts having appeared most frequently in publications on application of 3D printing in **orthopedic/prosthetic**

The top 15 concepts indexed from documents related to tissue/organs and orthopedic/prosthetic are shown in Figures 25 and 26, respectively. Concepts that frequently appeared in tissue/organs application include bioprinting, biocompatibility, bone formation/bone, pharmaceutical hydrogels, etc. This trend aligns with the bioprinting volume in tissue/organs application (**Figure 9d**). Cartilage fabrication (bone formation) is also the top subfield of tissue/organs (**Figure 21**). Pharmaceutical hydrogels (hydrogels) primarily contribute to this field as well (**Figure 22**). For orthopedic/prosthetic applications, concepts such as dental implants, bone formation, and artificial bone frequently appeared. This indicates that dental/bone-related innovation is the mainstream in orthopedic/prosthetic applications.

Conclusions & Outlook

In conclusion, the publication trends of the recent biomedical applications using 3D printing primarily contributed to journals over patents. The major techniques are the powder and extrusion methods using poly(lactic acid), polycaprolactone, and hydroxylapatite (HAp) as the materials. Tissue/organs engineering and pharmaceutical-based applications are the most attractive fields of biomedical applications. Cartilage, muscle, and skin are the top three with significant publications. Natural polymers have been widely studied for 3D printing and have been strongly favored over synthetic polymers as 3D printed hydrogels or in bioprinting.

There are several challenges with 3D printing tissue and organs. These include: (1) donor site morbidity and graft failure are still shortcomings faced; (2) stem cells' fragility and vulnerability that delay maturation of cells for time-sensitive treatment; (3) biomechanical limitations and functionality of the fabricated tissue; (4) short cell viability during bioprinting and after implantation; (5) disease from allografts and risk of body rejection; (6) biomaterials' ability to biodegrade within a desirable time frame and maintain structure. Advances in bioinks, media use, and application of stem cells have proven to make great strides in these shortcomings. 3D printing can also apply to surgical instrumentation such as hemostats, needle drivers, scalpel handles, retractors, and forceps are available in design packages such as SolidWorks. At this moment, the total journal publication number is dramatically higher than the corresponding patents. We can expect the commercialization of these innovative ideas in the foreseeable future.

Method

This work used data from the CAS Content Collection, which covers publications in more than 50,000 scientific journals from around the world in a wide range of disciplines, 62 patent authorities, and 2 defensive publications (Research Disclosures and IP.com).

The following search query was used to find as many documents related to biomedical applications of 3D printing as possible. About 22,000 documents were retrieved. Publications related to certain narrower fields or applications, or those involving certain substances, were obtained by searching within this entire document collection using corresponding search criteria.

((3d print? or three-dimensional print? or three-dimension print? or additive-manufactur? or stereolithog? or selective laser sintering? or fused deposition model? or direct metal laser sintering?) and (1/cc or 2/cc or 3/cc or 4/cc or 6/cc or 8/cc or 9/cc or 10/cc or 11/cc or 12/cc or 13/cc or 14/cc or 15/cc or 16/cc or 63/cc or 64/cc or 1/sx or 2/sx or 3/sx or 4/sx or 6/sx or 8/sx or 9/sx or 10/sx or 11/sx or 12/sx or 13/sx or 14/sx or 15/sx or 16/sx or 63/sx or 64/sx)) or (bioprint? or bioink? or bio-print? or bio-ink?)

Definition of Sections in CAS

Section 1: Pharmacology; Section 2: Mammalian Hormones; Section 3: Biochemical Genetics; Section 4: Toxicology; Section 6: General Biochemistry; Section 8: Radiation Biochemistry; Section 9: Biochemical Methods; Section 10: Microbial, Algal, and Fungal Biochemistry; Section 11: Plant Biochemistry; Section 12: Nonmammalian Biochemistry; Section 13: Mammalian Biochemistry; Section 14: Mammalian Pathological Biochemistry; Section 15: Immunochemistry; Section 16: Fermentation and Bioindustrial Chemistry; Section 63: Pharmaceuticals; Section 64: Pharmaceutical Analysis.

References

(1) González, C. M. *Infographic: The History of 3D Printing*. 2020. https://www.asme.org/topics-resources/content/infographic-the-history-of-3d-printing (accessed 11/1/2022).
(2) Agarwala, M.; Bourell, D.; Beaman, J.; Marcus, H.; Barlow, J. Direct selective laser sintering of metals. *Rapid Prototyping Journal* **1995**, *1* (1), 26–36. DOI: 10.1108/13552549510078113 (acccessed 2022/11/14).

(3) Körner, C. Additive manufacturing of metallic components by selective electron beam melting — a review. *International Materials Reviews* **2016**, *61* (5), 361-377. DOI: 10.1080/09506608.2016.1176289.
(4) Shirazi, S. F. S.; Gharehkhani, S.; Mehrali, M.; Yarmand, H.; Metselaar, H. S. C.; Adib Kadri, N.; Osman, N. A. A. A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. *Science and Technology of Advanced Materials* **2015**, *16* (3), 033502. DOI: 10.1088/1468-6996/16/3/033502.
(5) Derby, B. Inkjet Printing of Functional and Structural Materials: Fluid Property Requirements, Feature Stability, and Resolution. *Annual Review of Materials Research* **2010**, *40* (1), 395–414. DOI: 10.1146/annurev-matsci-070909-104502.
(6) Nikzad, M.; Masood, S. H.; Sbarski, I. Thermo-mechanical properties of a highly filled polymeric composites for Fused Deposition Modeling. *Materials & Design* **2011**, *32* (6), 3448–3456. DOI: 10.1016/j.matdes.2011.01.056.
(7) Tetsuka, H.; Shin, S. R. Materials and technical innovations in 3D printing in biomedical applications. *Journal of Materials Chemistry B* **2020**, *8* (15), 2930–2950, 10.1039/D0TB00034E. DOI: 10.1039/D0TB00034E.
(8) Lee, H.; Cho, D.-W. One-step fabrication of an organ-on-a-chip with spatial heterogeneity using a 3D bioprinting technology. *Lab on a Chip* **2016**, *16* (14), 2618-2625, 10.1039/C6LC00450D. DOI: 10.1039/C6LC00450D.
(9) Shim, J.-H.; Lee, J.-S.; Kim, J. Y.; Cho, D.-W. Bioprinting of a mechanically enhanced three-dimensional dual cell-laden construct for osteochondral tissue engineering using a multi-head tissue/organ building system. *Journal of Micromechanics and Microengineering* **2012**, *22* (8), 085014. DOI: 10.1088/0960-1317/22/8/085014.
(10) *Poly(Lactic Acid): Synthesis, Structures, Properties, Processing, and Applications*; 2010. DOI: 10.1002/9780470649848.
(11) Paxton, N. C.; Allenby, M. C.; Lewis, P. M.; Woodruff, M. A. Biomedical applications of polyethylene. *European Polymer Journal* **2019**, *118*, 412-428. DOI: 10.1016/j.eurpolymj.2019.05.037.
(12) Ng, W. L.; Goh, M. H.; Yeong, W. Y.; Naing, M. W. Applying macromolecular crowding to 3D bioprinting: fabrication of 3D hierarchical porous collagen-based hydrogel constructs. *Biomaterials Science* **2018**, *6* (3), 562-574, 10.1039/C7BM01015J. DOI: 10.1039/C7BM01015J.
(13) Green, D. W.; Lee, K. K.-H.; Watson, J. A.; Kim, H.-Y.; Yoon, K.-S.; Kim, E.-J.; Lee, J.-M.; Watson, G. S.; Jung, H.-S. High Quality Bioreplication of Intricate Nanostructures from a Fragile Gecko Skin Surface with Bactericidal Properties. *Scientific Reports* **2017**, *7* (1), 41023. DOI: 10.1038/srep41023.
(14) Aretxabaleta, M.; Xepapadeas, A. B.; Poets, C. F.; Koos, B.; Spintzyk, S. Comparison of additive and subtractive CAD/CAM materials for their potential use as Tübingen Palatal Plate: An in-vitro study on flexural strength. *Additive Manufacturing* **2021**, *37*, 101693. DOI: 10.1016/j.addma.2020.101693.
(15) Ngo, T. T.; Hoffman, L.; Hoople, G. D.; Trevena, W.; Shakya, U.; Barr, G. Surface morphology and drug loading characterization of 3D-printed methacrylate-based polymer facilitated by supercritical carbon dioxide. *The Journal of Supercritical Fluids* **2020**, *160*, 104786. DOI: 10.1016/j.supflu.2020.104786.
(16) Kwon, J.-S.; Kim, J.-Y.; Mangal, U.; Seo, J.-Y.; Lee, M.-J.; Jin, J.; Yu, J.-H.; Choi, S.-H. Durable Oral Biofilm Resistance of 3D-Printed Dental Base Polymers Containing Zwitterionic Materials. *International Journal of Molecular Sciences* **2021**, *22* (1), 417.
(17) Ho, T.-C.; Chang, C.-C.; Chan, H.-P.; Chung, T.-W.; Shu, C.-W.; Chuang, K.-P.; Duh, T.-H.; Yang, M.-H.; Tyan, Y.-C. Hydrogels: Properties and Applications in Biomedicine. *Molecules* **2022**, *27* (9), 2902.
(18) Bertassoni, L. E.; Cecconi, M.; Manoharan, V.; Nikkhah, M.; Hjortnaes, J.; Cristino, A. L.; Barabaschi, G.; Demarchi, D.; Dokmeci, M. R.; Yang, Y.; et al. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab on a Chip* **2014**, *14* (13), 2202–2211. DOI: 10.1039/C4LC00030G.

(19) Hallensleben, M. L.; Fuss, R.; Mummy, F. Polyvinyl Compounds, Others. In *Ullmann's Encyclopedia of Industrial Chemistry*, pp 1–23.
(20) van der Heide, D.; Cidonio, G.; Stoddart, M. J.; D'Este, M. 3D printing of inorganic-biopolymer composites for bone regeneration. *Biofabrication* **2022**, *14* (4), 042003. DOI: 10.1088/1758-5090/ac8cb2.
(21) Malafaya, P. B.; Reis, R. L. Bilayered chitosan-based scaffolds for osteochondral tissue engineering: Influence of hydroxyapatite on in vitro cytotoxicity and dynamic bioactivity studies in a specific double-chamber bioreactor. *Acta Biomaterialia* **2009**, *5* (2), 644–660. DOI: https://doi.org/10.1016/j.actbio.2008.09.017.
(22) Ng, W. L.; Yeong, W. Y.; Naing, M. W. Development of Polyelectrolyte Chitosan-gelatin Hydrogels for Skin Bioprinting. *Procedia CIRP* **2016**, *49*, 105–112. DOI: 10.1016/j.procir.2015.09.002.
(23) Dai, L.; Cheng, T.; Duan, C.; Zhao, W.; Zhang, W.; Zou, X.; Aspler, J.; Ni, Y. 3D printing using plant-derived cellulose and its derivatives: A review. *Carbohydrate Polymers* **2019**, *203*, 71–86. DOI: 10.1016/j.carbpol.2018.09.027.
(24) Chinga-Carrasco, G. Potential and Limitations of Nanocelluloses as Components in Biocomposite Inks for Three-Dimensional Bioprinting and for Biomedical Devices. *Biomacromolecules* **2018**, *19* (3), 701–711. DOI: 10.1021/acs.biomac.8b00053.
(25) Palmieri, V.; Lattanzi, W.; Perini, G.; Augello, A.; Papi, M.; De Spirito, M. 3D-printed graphene for bone reconstruction. *2D Materials* **2020**, *7* (2), 022004. DOI: 10.1088/2053-1583/ab6a5d.
(26) Wang, W.; Huang, B.; Byun, J. J.; Bártolo, P. Assessment of PCL/carbon material scaffolds for bone regeneration. *Journal of the Mechanical Behavior of Biomedical Materials* **2019**, *93*, 52–60. DOI: 10.1016/j.jmbbm.2019.01.020.
(27) López Marzo, A. M.; Mayorga-Martinez, C. C.; Pumera, M. 3D-printed graphene direct electron transfer enzyme biosensors. *Biosensors and Bioelectronics* **2020**, *151*, 111980. DOI: 10.1016/j.bios.2019.111980.
(28) Zhai, Z.; Sun, J. Research on the low-temperature degradation of dental zirconia ceramics fabricated by stereolithography. *The Journal of Prosthetic Dentistry* **2021**. DOI: 10.1016/j.prosdent.2021.11.012.
(29) Meischel, M.; Eichler, J.; Martinelli, E.; Karr, U.; Weigel, J.; Schmöller, G.; Tschegg, E. K.; Fischerauer, S.; Weinberg, A. M.; Stanzl-Tschegg, S. E. Adhesive strength of bone-implant interfaces and in-vivo degradation of PHB composites for load-bearing applications. *Journal of the Mechanical Behavior of Biomedical Materials* **2016**, *53*, 104–118. DOI: 10.1016/j.jmbbm.2015.08.004.
(30) Abdullah, A. M.; Rahim, T. N. A. T.; Hamad, W. N. F. W.; Mohamad, D.; Akil, H. M.; Rajion, Z. A. Mechanical and cytotoxicity properties of hybrid ceramics filled polyamide 12 filament feedstock for craniofacial bone reconstruction via fused deposition modelling. *Dental Materials* **2018**, *34* (11), e309–e316. DOI: 10.1016/j.dental.2018.09.006.
(31) Suwa, M.; Kirihara, S.; Sohmura, T. Fabrication of Alumina Dental Crown Model with Biomimetic Structure by Using Stereolithography. In *Advanced Processing and Manufacturing Technologies for Structural and Multifunctional Materials IV*, 2010; pp 239–245.
(32) Lee, J. S. Photo-curable resin compositions and method of using the same in three-dimensional printing for manufacturing artificial teeth and denture base. US20140167300, A1 2014.
(33) O'Neill, B. *Ultimate guide to silicone 3D printing*. 2022. https://www.aniwaa.com/guide/3d-printers/silicone-3d-printing/ (accessed 11/1/2022).
(34) Gilev, M. V.; Bazarny, V. V.; Volokitina, E. A.; Polushina, L. G.; Maksimova, A. Y.; Kazakova, Y. E. Laboratory Monitoring of Bone Tissue Remodeling after Augmentation of Impression Intraarticular Fracture with Different Types of Bone Graft. *Bulletin of Experimental Biology and Medicine* **2019**, *167* (5), 681–684. DOI: 10.1007/s10517-019-04598-7.

(35) Islam, M.; Sadaf, A.; Gómez, M. R.; Mager, D.; Korvink, J. G.; Lantada, A. D. Carbon fiber/microlattice 3D hybrid architecture as multi-scale scaffold for tissue engineering. *Materials Science and Engineering: C* **2021**, *126*, 112140. DOI: 10.1016/j.msec.2021.112140.
(36) Hussain, M.; Kumar, V.; Mandal, V.; Singh, P. K.; Kumar, P.; Das, A. K. Development of cBN reinforced Ti6Al4V MMCs through laser sintering and process optimization. *Materials and Manufacturing Processes* **2017**, *32* (14), 1667–1677. DOI: 10.1080/10426914.2017.1303152.
(37) Lim, H.-K.; Choi, Y.-J.; Choi, W.-C.; Song, I.-S.; Lee, U.-L. Reconstruction of maxillofacial bone defects using patient-specific long-lasting titanium implants. *Scientific Reports* **2022**, *12* (1), 7538. DOI: 10.1038/s41598-022-11200-0.
(38) Kim, D.-H.; Tsui, J. H. Y. Electroconductive decellularized extracellular matrix compositions for preparation of engineered tissues and related methods. US20210108180 A1, 2021.
(39) Brett Kotlus. 3D design and fabrication system for implants. US9250620 B2, 2015.
(40) Groll, J.; Boland, T.; Blunk, T.; Burdick, J. A.; Cho, D.-W.; Dalton, P. D.; Derby, B.; Forgacs, G.; Li, Q.; Mironov, V. A.; Moroni, L.; Nakamura, M.; Shu, W.; Takeuchi, S.; Vozzi, G.; Woodfield, T. B. F.; Xu, T.; Yoo, J. J.; Malda, J., Biofabrication: reappraising the definition of an evolving field. *Biofabrication* **2016,** *8* (1). DOI: 10.1088/1758-5090/8/1/013001.
(41) Zhang, Y. S.; Yue, K.; Aleman, J.; Mollazadeh-Moghaddam, K.; Bakht, S. M.; Yang, J.; Jia, W.; Dell'Erba, V.; Assawes, P.; Shin, S. R.; Dokmeci, M. R.; Oklu, R.; Khademhosseini, A., 3D Bioprinting for Tissue and Organ Fabrication. *Annals of Biomedical Engineering* **2016,** *45* (1), 148–163. DOI: 10.1007/s10439-016-1612-8.
(42) Roseti, L.; Cavallo, C.; Desando, G.; Parisi, V.; Petretta, M.; Bartolotti, I.; Grigolo, B., Three-Dimensional Bioprinting of Cartilage by the Use of Stem Cells: A Strategy to Improve Regeneration. *Materials* **2018,** *11* (9). DOI: 10.3390/ma11091749.
(43) Koons, G. L.; Diba, M.; Mikos, A. G., Materials design for bone-tissue engineering. *Nature Reviews Materials* **2020,** *5* (8), 584–603. DOI: 10.1038/s41578-020-0204-2.
(44) Seyedmahmoud, R.; Çelebi-Saltik, B.; Barros, N.; Nasiri, R.; Banton, E.; Shamloo, A.; Ashammakhi, N.; Dokmeci, M. R.; Ahadian, S., Three-Dimensional Bioprinting of Functional Skeletal Muscle Tissue Using GelatinMethacryloyl-Alginate Bioinks. *Micromachines* **2019,** *10* (10). DOI: 10.3390/mi10100679.
(45) Yang, G. H.; Kim, W.; Kim, J.; Kim, G., A skeleton muscle model using GelMA-based cell-aligned bioink processed with an electric-field assisted 3D/4D bioprinting. *Theranostics* **2021,** *11* (1), 48–63. DOI: 10.7150/thno.50794.
(46) Gungor-Ozkerim, P. S.; Inci, I.; Zhang, Y. S.; Khademhosseini, A.; Dokmeci, M. R., Bioinks for 3D bioprinting: an overview. *Biomaterials Science* **2018,** *6* (5), 915–946. DOI: 10.1039/c7bm00765e.
(47) Noor, N.; Shapira, A.; Edri, R.; Gal, I.; Wertheim, L.; Dvir, T., 3D Printing of Personalized Thick and Perfusable Cardiac Patches and Hearts. *Advanced Science* **2019,** *6* (11). DOI: 10.1002/advs.201900344.
(48) Gaebel, R.; Ma, N.; Liu, J.; Guan, J.; Koch, L.; Klopsch, C.; Gruene, M.; Toelk, A.; Wang, W.; Mark, P.; Wang, F.; Chichkov, B.; Li, W.; Steinhoff, G., Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. *Biomaterials* **2011,** *32* (35), 9218–9230. DOI: 10.1016/j.biomaterials.2011.08.071.
(49) Singh, D.; Singh, D.; Han, S., 3D Printing of Scaffold for Cells Delivery: Advances in Skin Tissue Engineering. *Polymers* **2016,** *8* (1). DOI: 10.3390/polym8010019.
(50) Azad, M. A.; Olawuni, D.; Kimbell, G.; Badruddoza, A. Z. M.; Hossain, M. S.; Sultana, T., Polymers for Extrusion-Based 3D Printing of Pharmaceuticals: A Holistic Materials–Process Perspective. *Pharmaceutics* **2020,** *12* (2). DOI: 10.3390/pharmaceutics12020124.
(51) ten Kate, J.; Smit, G.; Breedveld, P., 3D-printed upper limb prostheses: a review. *Disability and Rehabilitation: Assistive Technology* **2017,** *12* (3), 300–314. DOI: 10.1080/17483107.2016.1253117.
(52) Ventola, C. L., Medical Applications for 3D Printing: Current and Projected Uses. *P T* **2014,** *39* (10), 704–11.

(53) Park, J. W.; Kang, H. G., Application of 3-dimensional printing implants for bone tumors. *Clinical and Experimental Pediatrics* **2022,** *65* (10), 476–482. DOI: 10.3345/cep.2021.01326
(54) Vorndran, E.; Moseke, C.; Gbureck, U., 3D printing of ceramic implants. *MRS Bulletin* **2015,** *40* (2), 127–136. DOI: 10.1557/mrs.2015.326.
(55) Bhattacharjee, N.; Urrios, A.; Kang, S.; Folch, A., The upcoming 3D-printing revolution in microfluidics. *Lab on a Chip* **2016,** *16* (10), 1720–1742. DOI: 10.1039/c6lc00163g.
(56) Prabhakar, P.; Sen, R. K.; Dwivedi, N.; Khan, R.; Solanki, P. R.; Srivastava, A. K.; Dhand, C., 3D-Printed Microfluidics and Potential Biomedical Applications. *Frontiers in Nanotechnology* **2021,** *3*. DOI: 10.3389/fnano.2021.609355.
(57) Tajeddin, A.; Mustafaoglu, N., Design and Fabrication of Organ-on-Chips: Promises and Challenges. *Micromachines* **2021,** *12* (12). DOI: 10.3390/mi12121443.
(58) Parr, W. C. H.; Burnard, J. L.; Wilson, P. J.; Mobbs, R. J., 3D printed anatomical (bio)models in spine surgery: clinical benefits and value to health care providers. *Journal of Spine Surgery* **2019,** *5* (4), 549–560. DOI: 10.21037/jss.2019.12.07.