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Last technological advancement in additive manufacturing for cardiovascular applications

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Abstract. **Additive manufacturing (AM) has increasing applications in medicine in recent times. This technology has emerged in cardiovascular medicine as an intelligent system for the improvement of medical devices, the preparation of patient-specific models, and the prototyping of grafts. This review traces the research and development in the production of surgical guides and synthetic grafts for cardiac and vascular applications over the last few years. It also traces the recent widespread use of 3D-printed specific-patient models for cardiovascular surgical interventions. A current view of AM strategies, materials and solutions to improve cardiovascular patient outcomes is also provided.**

Key Words:
Additive manufacturing, 3D Printing, Surgical guide, Model, Cardiac patch, Stent, Vascular graft.

Introduction

Although the number of transplants increases every year, the transplantation of organs and tissues suffers from limited donor supply. This gap can be circumvented, at least to some extent, through tissue engineering and regenerative medicine. Tissue engineering and regenerative medicine are multidisciplinary sciences, which aim is to develop biological substitutes that can maintain, improve, or restore tissue function[1,2]. Regenerative medicine encompasses numerous strategies and involves different materials and cells, as well as various combinations thereof. The purpose of regenerative medicine is to use biomaterial scaffolds that mimic the natural extracellular matrix (ECM) in support of 3D cell growth to restore cell behavior and tissue function. Biomaterials should support the innate abilities of cells to sense their environment through cell-to-cell and cell-to-ECM contacts, assemble complex biological networks, and respond naturally to promote tissue healing and regeneration. In addition, an ideal scaffold should be biocompatible, immunologically inert, biodegradable, porous for ensuring vascularization and cell migration[3]. Several biomaterials, both natural and synthetic, can be 3D-printed. Natural materials are components of ECM, polymers extracted from decellularized tissues or plants, such as gelatin, alginate, fibroin, agarose, collagen, and hyaluronic acid[4]. These natural materials are biocompatible and biodegradable and behave similarly to the healthy ECM. Hydrophilicity, cell affinity, and the preservation of native integrin-binding sites are additional advantages of natural materials[5]. Synthetic biomaterials include polymers such as polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), and a combination of them, as well as ceramic materials including hydroxyapatite and calcium phosphate. Synthetic materials have received increasing attention due to their controllable biocompatibility, biodegradability, and chemical properties. Their architecture, mechanical properties, and degradation rate can be precisely tuned to a specific application. They can be engineered with peptides to improve cell affinity and consequently promote cell infiltration. In addition, synthetic materials prevent batch-to-batch variations[6].

In the past decade, additive manufacturing (AM) has emerged as a versatile technology platform for customized manufacturing of synthetic substitutes for regenerative medicine. AM produces physical objects from a digital 3D design file. Computer-aided design (CAD) software create virtual 3D design file using 3D modeling program, 3D scanner, or medical scanning techniques such as computed tomography (CT) or magnetic resonance

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imaging (MRI). The CAD data are then converted into multiple 2D cross-section layers. Following the predefined 2D pattern, a 3D printer manufactures a 3D structure[7]. To date, various AM methodologies have been developed to enable 3D printing of various natural and synthetic materials, but also responsive materials. These particular materials are capable of self-transformation into predefined shapes in response to micro-environmental stimuli to perform predefined functions. When responsive materials are subjected to appropriate shifts in temperature, solvent, humidity, light, pH, electricity, or magnetic field exhibit intelligent behavior, including shape memory, self-assembly, self-actuation, self-sensing, and self-healing. Thus, the exposure to physical or chemical stimuli mediates a predetermined time-dependent change. This implies that the 3D-printed structure must exhibit at least two stable configurations before and after the application of the triggering stimulus[8]. The introduction of the fourth dimension, i.e., time, in AM technology is termed as "4D printing". Stimuli-responsive polymers have functional groups along the polymer backbone that are sensitive to changes in state. The main advance of stimulus-responsive polymers is the reversibility of the response, even if reversibility introduces drawbacks during printing procedure. To overcome these issues, stimulus-responsive polymers can be combined with other nonresponsive polymers or ceramics that function as biological or mechanical property enhancers or processing aids[9]. For tissue engineering purposes, temperature-responsive polymers are frequently selected, especially when temperature changes can be applied and controlled in a noninvasive way (e.g., exposure to 37 °C, the human body temperature). Shape memory polymers (SMPs) and responsive polymer solutions are currently the most common temperature-responsive materials available. 3D-printed SMPs for tissue engineering purposes include soybean oil epoxidized acrylate[10], PCL triol[11], poly(ether-ester-urethane)[12], and PCL dimethacrylate[13]. For instance, PCL dimethacrylate was 3D-printed to manufacture a thermally actuated tracheal stent. When in contact with body temperature, the stent expanded to fit the tracheal anatomy after being positioned into the tracheal section, preventing any potential injury during insertion[13]. Reactive polymer solutions are typically copolymers that exhibit a critical temperature that influences hydrophobic and hydrophilic interactions with the solvent. Therefore, a change in temperature causes a modification in inter- and intramolecular interactions, resulting in the precipitation of polymers and expansion or shrinkage of hydrogels. Some reactive polymer solutions that are utilized in tissue engineering and drug delivery applications are poly N-vinylcaprolactam[14], pluronic hydrogel[15], and gelatin-methacryloyl (GelMA)[16]. For instance, a self-standing small-diameter vasculature was printed combining GelMA and catechol. The composite material underwent rapid oxidative cross-linking *in situ* to form an elastic hydrogel with controllable mechanical strength, high cell and tissue adhesion and functionalization. The printed vascular construct showed biomimetic characteristics such as proper biomechanics, tissue affinity, and *in vivo* autonomous connection and vascular remodeling[16].

The growing use of AM in medicine hinges on its distinguishing advantages, including design freedom, accuracy, personalization, and timesaving. In contrast to traditional methods of production, AM ensures customization of medical devices, equipment, and drugs, increased productivity, specificity and cost-effectiveness. AM can be used to create personalized prosthetics, implants, and anatomical models, to reconstruct organs and tissues, and to manufacture medical instruments. Furthermore, AM can be harnessed for researching pharmaceutical dosage forms, drug delivery system, and innovative medical device[17]. Personalized 3D-printed implants are manufactured to fit the anatomy or other requirements of a specific single patient. By monitoring several parameters during the design phase, AM technology can control the implant macrogeometry to perfectly match tissue defects and its microarchitecture to guarantee sufficient porosity and interconnectivity for improving cell transportation and nutrient diffusion. Through an elevated degree of control, localization of biomolecular cues, and tailoring mechanical properties, AM can create complex material geometries that resemble endogenous tissues and exhibit analogous mechanical properties[18]. For instance, AM has been used for the production of patient-specific skin[19,20], bone[21], vascular grafts[22], cardiac tissue[23,24], tracheal splints[25], and cartilaginous structures[26,27]. AM can produce prosthetics for organ replacements and orthotics for external deformity correction. Prosthetics for limbs, hands, fingers and eyes have already been designed for AM production[28-30]. Multi-material jetting printers can mix multicolor materials to achieve functionality, comfort, and esthetics. Additionally, simplified designs make prosthetics accessible to people with limited access to healthcare services and to children who rapidly outgrow prostheses[31].

Patient-specific models can be 3D-printed to facilitate the understanding of patient's pathological anatomy and support precise preoperative planning. 3D-printed models can reproduce the size, weight and texture of internal organs or anatomical parts, allowing surgeons to rehearse complicated procedures. Customized models can support the pre-identification of precise surgical cuts and implant placement to reduce surgery time and to help in the best approach evaluation to restore anatomical features after surgery. Additionally, after sterilization, printed models can be moved in the operating room for guiding intraoperative procedures[32]. 3D-printed models for planning surgery have already been used for the management of several operations, such as cardiac[33], spinal[34], cranial[35], or renal surgery[36]. Moreover, 3D printers can be used to produce surgical instruments including scalpel, hemostats, clamps and forceps. 3D-printed surgical instruments provide numerous advantages, such as high design flexibility, limited production cost and minimal production time[37].

The research and development of new pharmaceutical forms as well as drug production can be performed by AM technologies. The US Food and Drug Administration approved the first 3D-printed pharmaceutical product, an orodispersible tablet supporting complex and customized dosage of the antiepileptic levetiracetam, in 2016[38]. The AM drug delivery system enables the precise control of the spatial distribution of active pharmaceutical elements, the deposition of very small amounts of active principles, and the production of complex geometries. In addition, AM allows the rapid fabrication of varying compositions for screening activities or preparation of personalized dose strengths[39].

In this review, we will report the most important technological advances of AM in cardiovascular medicine over the last years. The benefits and challenges that currently limit AM application for cardiac and vascular purposes as well as the possible strategies for overcoming these topics will be discussed.

Patient-Specific Anatomical Models

In cardiovascular medicine, anatomical models are generally created as teaching tools for surgical planning or medical device design and prototyping[40]. Before cardiac surgery, patients routinely undergo pre-procedural imaging (CT scan or MRI). However, defining the anatomical features significant to a planned cardiac surgery is extremely challenging and time consuming. Although current modeling software has semi-automatic segmentation algorithms for cardiac structures, complex segmentations of a whole heart, including the individual internal structures, can still take several hours. 3D-printed models can play a critical role in surgical planning for the treatment of advanced heart diseases, including those involving a heart with unique anatomical features that are difficult to interpret from flat CT or MRI data[41]. In recent years, 3D printed preoperative models have been tested to better address different types of cardiovascular surgery such as congenital heart disease correction, congenital aortic anomalies correction, and tricuspid valve disease. Shearn et al[42] produced 3D models to complement imaging data for a 12-month-old child with double-outlet right ventricle and two ventricular septal defects (VSD). White rigid and flexible color models were produced to support case management and surgical planning. The models were discussed both at a joint multidisciplinary meeting and between the surgeon and the cardiologist. From the blood pool model, clinicians were able to determine that the position of the coronary arteries meant an arterial switch operation was unlikely to be feasible. The soft myocardium model enabled clinicians to evaluate the VSD anatomy and relationship with the aorta[42]. Along similar lines, Sun et al[43] described a protocol for modeling the CT images of congenital aortic anomalies into 3D data and for printing an anatomically realistic 3D model. Complex congenital aortic anomalies include diverse types of malformations that may be clinically asymptomatic or present with respiratory or esophageal symptoms. Using this protocol, surgeons identify the vessel location of complex aortic anomalies, which is helpful for preoperative planning and intraoperative guidance[43]. Santoro et al[44] reported three cases of ascending aorta pseudoaneurysm (AAP) successfully treated by transcatheter closure after the pre-surgical planning with 3D-printed models. AAP is a rare and potentially lethal complication of surgical interventions, atherosclerosis, or infections. It may remain clinically silent until involvement of the surrounding structures, or rupture. The transcatheter option is emerged as a safer and cost-effective alternative to surgical repair. However, this approach is still technically demanding because of the unpredictability and complexity of local

anatomy and the lack of dedicated devices. The site, size, and shape of the feeding breach and its spatial relationship with the neighboring structures was finely detail using 3D-printed models. This approach added several advantages to simple 3D digital reconstruction, including appreciating the spatial relationship between the cardiac and extracardiac structures, mimicking *ex vivo* the planned interventional procedure, testing different types of devices, and consequently decreasing entire procedure time and overall risk of the treatment[44].

Harb et al[45] presented four cases of primary tricuspid valve (TV) disease where AM was crucial for pre-operative planning, patients understanding of disease and planned operation. Four patients, both men and women aged 20 to 70, underwent 3D transesophageal echocardiography and contrast-enhanced 4D CT. Then, imaging-processing software delineated and segmented the right-sided structures, including the TV annulus and leaflets. Patient specific 3D models were then printed using soft material for valve leaflets and right-sided chambers, and rigid material for the pacemaker leads when required. Given the rarity of isolated primary TV disease and the limited surgical experience, this approach was pivotal for an exhaustive pre-procedural planning and the intervention success. Volumetric imaging and 3D printing allowed an improved understanding TV anatomy, including its position in the chest, the number and size of leaflets, the size of the annulus and the right ventricle[45].

In the last few years, 3D-printed anatomical models have also been widely used for subclavian artery percutaneous vascular interventions[46], carotid artery stenting[47], saphenous vein grafting[48], and pulmonary arteriovenous malformations surgery[49].

Surgical Guides

As the preoperative models, also the surgical guides have had a greater development and innovation due to the introduction of the AM. Patients with high or moderate risk for surgical valve replacement and with severe symptomatic aortic stenosis are generally treated with trans-catheter aortic valve replacement (TAVR). Despite its widespread use, a recurring challenge of TAVR is achieving a personalized prosthetic valve fit for every patient. Poor fit may result from unanticipated interactions between the geometry and placing of a prosthetic valve and the patient anatomy and may lead to considerable complications. Oversizing the prosthetic valve can lead to annular rupture and under sizing can result in an ineffective paravalvular seal or prosthetic embolization. Actually, it is still difficult perform the prediction on how the prosthetic valve will fit with the unique individual's anatomy. In a recent study, Hosny et al[50] employed AM to develop and validate a benchtop workflow to test physical interactions between an expandable custom-designed TAVR valve sizer and patient-specific aortic root anatomy, including the active displacement of calcified aortic leaflets during prosthetic valve opening. This workflow has the potential to guide the selection of optimal valve size for a given patient and alert the physician to the presence of unfavorable geometric interactions between the patient anatomy and the artificial valve that could result in procedural complications. Through further feedback with real patient data, this pipeline also can offer prospects for a better understanding of the mechanisms and factors underlying successful percutaneous valve placement and may help inform future valve designs at sites other than the aorta[50].

A surgical guide for the bypass placement during coronary artery bypass graft (CABG) was developed and 3D-printed by Cappello et al[51]. They presented the entire workflow for the production of the patient-specific 3D-printed surgical guide from data acquisition and image segmentation to final prototyping. The surgical guide aims to select the proper areas for bypass placement and to recognize stenosis reducing the procedural time. The 3D-printed surgical guide was tested on a 3D heart phantom. The physical surgical guide complied with the virtual model, according to its dimensions. A fitting test provided the correct positioning of the surgical guide on the heart phantom with desired flexibility and stability. The inner surface in contact with the epicardium was smooth and without excessive roughness, whereas the outer surface was rigid and resistant to damage from medical instrumentation used during CABG surgery. Meanwhile, surgical holes marked the regions of interest allowing the identification of the correct target for bypass placement[51].

Brzeziński et al[52] proposed a novel holdfast device for left atrial appendage (LAA). The LAA is a small, finger-like extension of the left atrium, and its exclusion is used as a treatment strategy to prevent ischemic stroke. A polyamide LAA exclusion device was implanted in the heart of

porcine model, and histological responses to the device were assessed. Complete occlusion was seen without any pathological findings during the incubation time. The surface of the atrium under the holdfast device was smooth and without clots. The foreign body reaction to the LAA holdfast device was comparable to that to control. Thus, the 3D-printed device appeared to fulfill desirable biocompatibility[52].

Cardiac Tissue Restoration and Cardiac Tissue Models

Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide. Adult cardiac muscle is presumed to lack self-repair and regenerative ability. Organ donors are limited, and the cellular therapies are not completely satisfactory. Indeed, the direct injection of cardiomyocytes into the epicardial infarct zone showed a limited capacity of engraft and restoration of cardiac functions. Traditional cardiac patches guarantee a temporary mechanical support to prevent the progression of post-infarction left ventricular remodeling, but they are ineffective in regeneration[53]. For overcoming the scarce myocardial restoration of traditional patches, several strategies have been tested, such as the addition of stem cell extracellular vesicles as regenerative biological signals[54]. Recently, 4D printing was investigated to improve cardiac patch integration. These novel patches can change shape on-demand to mimic and recreate the curvature of heart surface and to support the adhesion and growth of cells guarantying the vascularization and engraftment. Cui et al[55] printed 4D cardiac patches reproducing the architectural and biological features of native myocardium using an ink consisting of gelatin methacrylate and polyethylene glycol diacrylate. The patches were printed to form a flat 3D pattern that become a 4D curved architecture as a result of printing parameters, water content, swelling, and ionic strength. The smart patches provided mechanical support as well as physiologically tunable structure with an elastic and bioactive matrix for cell implantation. The patches exhibited high levels of cell engraftment and vascularization in MI mouse model[55]. Instead, Wang et al[56] fabricated 4D near-infrared (NIR) light-sensitive cardiac patches with aligned microstructure and adjustable curvature. The ink material consisted of thermally responsive shape memory polymer and graphene to achieve the NIR-responsive 4D transformation. The NIR-responsive 4D constructs were able to actuate a dynamic and remotely controllable spatiotemporal transformation. The microgrooves on construct surface were optimized to ensure uniform and aligned cell distribution and myocardial maturation on 4D curved cardiac constructs[56].

Asulin et al[57] proposed a different regenerative strategy based on the introduction of electronic component in the engineered cardiac construct. They fabricated 3D printed heart tissue in a single step with integrated electronics. For the first time, an electronic system was printed under physiological conditions, i.e., 37°C and aqueous solution, inside an engineered tissue. To do this, they used three different bio-inks: the cellular ink containing ECM-based hydrogel and neonatal rat ventricular cardiac cells, the second ink containing the conductive material (graphite flakes) of the electronic system dispersed in liquid polydimethylsiloxane, the third ink contained the dielectric material covering the electrodes. The three bio-inks were loaded into three separated extrusion cassettes and simultaneously 3D-printed to produce the electronic cardiac patch. The engineered tissue was able to contract, meanwhile, the electronics can sense its function and provide electrical stimulation for pacing. Therefore, the system would allow to monitor the function of the engineered tissue after transplantation and, when necessary, to intervene effectively at a distance[57].

The fabrication of 3D cardiac tissue is required not only for regenerative aims, but also for drug discovery and screening. Alonzo et al[58] developed a 3D bio printed cardiac tissue model with sustained longevity and function by mixing cardiac cells with a gel-based matrix. The model contains cardiomyocytes, cardiac fibroblasts, and endothelial cells. The introduction of all cardiac cell types was crucial to improve the fidelity of the tissue-engineered myocardium as cardiac fibroblasts secrete and regulate the ECM providing mechanical support, electrical conduction, and paracrine signaling, while endothelial cells form vessels and perform paracrine activity. The cardiac cells were dispersed in a bio-ink composed of alginate, guluronic acid and gelatin. The 3D-printed scaffolds had circular waffle-like structure with striated texture to resemble the actual cardiac tissue. The cardiac scaffolds with highly interconnected pores promoted long-term cell viability, function, and phenotype maintenance over a 21-day culture period. The 3D model mimicking the anisotropic structure of actual cardiac tissue can be a versatile tool for analyzing cell behavior and function for

various applications such as drug screening and tissue engineering[58]. Furthermore, scaffold-free 3D tubular cardiac constructs were designed for screening cardiotoxicity and cardiac response of new drugs[59]. Precisely, cardiomyocytes derived from induced pluripotent stem cells, endothelial cells and fibroblasts were combined in cardiac spheroids, and then 3D printed on needle array. The cellular organization in the cardiac constructs was similar to that observed during organ transplantation. The constructs were responsive to electrical stimulation and returned to their initial beat rate after stimulation was stopped[59]. By analyzing changes in needle tip movement, the contractile force of the cardiac constructs can be evaluated after stimulation with a different drug[60]. As drug response and cardiotoxicity was dependent on the type of applied drug, this analysis method can be applied during the development and screening of new drug[60].

Vascular Stent and Vascular Graft

The procedures of revascularization comprise angioplasty, placement of stents and vascular grafting. Vascular stents are hollow mesh tubes implanted within the blood vessel to support the wall and prevent vessel closure. Some of them are called drug-eluting stents as slowly release drugs that block cell proliferation to prevent vessel obstruction. However, endovascular therapy remains uncertain because of recurrence, local thrombus formation and restenosis due to the adhesion of blood platelets and the migration and proliferation of smooth muscle cells. Bioabsorbable stents (BRSs) can overcome these drawbacks as they gradually disappear after reendothelization while still guaranteeing the support function. Lu et al[61] 3D printed a novel BRS made of poly (p-dioxanone) polymers and bis-(2,6-diisopropylphenyl) carbodiimide. The thickness and vessel coverage of the 3D-printed BRS were controlled by setting printing parameters. The attachment and growth of endothelial cell were confirmed by *in vitro* experiments. As dimension, diameter, and width of the stent can be regulated during the design phase, this system can be a versatile method for manufacturing intravascular BRSs for small or large vessels[61]. While the vascular stent with an internal diameter greater than 6 mm has been broadly developed, also with AM technique[62], the small diameter peripheral vascular stent remains a challenge. Biodegradable SMPs seem to be good candidates for the development of novel small-caliber stents. Biodegradable stent with shape memory effect could reduce the possibility of thrombosis and embolism, attenuate the lesions of the surgical approach improving recovery. A stent with high mechanical properties and shape memory effect was printed using a bio-ink made of PCL and the oligosaccharide β-cyclodextrin (βCD)[63]. The 4D printed stent showed good biocompatibility, suitable degradation rate, and drug-sustained release. Due to the presence of βCD, the wettability and biocompatibility of the materials are improved, as demonstrated by endothelial cell adhesion and proliferation. Due to the shape memory effect, the stent can be implanted within compacted small size vessel, restore the 3D-printed shape after deployment under thermal stimulus, and then withstand high blood flow and pressure under physiological vascular conditions. Furthermore, the sustained long-term paclitaxel release from the 4D-printed stent is expected to solve the restenosis caused by intimal hyperplasia of traditional stents for small-diameter vessels disease[63].

The recent research and development in 4D-printed stents has also concerned their degradation profile. The degradation of 4D-printed PLA stents was investigated[64] in dynamic condition after self-expandable deployment. Precisely, the stents were implanted in simulated blood vessels with minimal microstructural damage at 60°C followed by 8-week degradation tests. The microstructure damage caused by deployment accelerated the degradation of stents faster than fluid shear stress. Furthermore, the relationship between stent injury, vascular injury, and stent deployment temperature was demonstrated by microstructural analysis and numerical simulation on the stent by finite element analysis. It is believed that this new evidence on degradation behavior could minimize the medical risk of 4D-printed stents[64].

Vascular grafts are used in bypass surgery to replace the damaged native blood vessels in the treatment of severe peripheral and cardiovascular disease. Artificial polymeric grafts offer several advantages including flexible shape, length, and diameter. Among biopolymers, thermoplastic polyurethane (TPU) has ideal elastic and mechanical properties and essential blood compatibility to make it a good candidate for vascular graft production. Martin et al[65] combined TPU with rifampicin (RIF) or dipyridamole (DIP), an antibiotic and an antiplatelet drug, respectively. The resulting materials demonstrated good hemocompatibility and cytocompatibility, drug release, and

proliferation of endothelial cells. Vascular grafts containing both drugs were 3D printed by loading DIP-TPU and RIF-TPU in a dual extrusion fused deposition modelling printer[65].

Biodegradable polymers are also investigated for vascular grafting as they can degrade and replace native vascular functionality. PCL is broadly used in vascular grafting due to its non-toxicity and biocompatibility[66]. For instance, PCL was properly mixed with DIP to reduce smooth muscle cell proliferation and promote proliferation of vascular endothelial cells. Then, biodegradable PCL-based vascular grafts loaded with DIP were successfully manufactured using AM technology[67]. *In vitro* tests demonstrated that DIP-PCL grafts were able to bear linear drug release for 30 days, and that the number of deposited platelets on material surface was inversely proportional to the amount of DIP loaded in the grafts[67].

Conclusions

In this review, we have presented the current technological advancement of AM in cardiovascular medicine. Several reports have documented the advantages brought by 3D-printed patient-specific anatomical models in the outcome of problematic surgical interventions due to the lack of previous experience, surgery complexity, or hard surgical case. The improvements in the design and manufacturing of surgical guides were also highlighted, but the future introduction of new combinations of materials and printing devices will certainly expand their design possibilities.

The cardiac tissue restoration still represents a goal to be completely achieved despite recent studies on 4D printing of responsive biomaterials, organoid bioprinting, and electronic cardiac patches. Meanwhile, the innovation in vascular stent and graft design has currently benefited from the 3D printing of bioabsorbable materials and shape memory polymers.

Conflict of Interest
The authors declare that they have no conflict of interests.

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