Additive Manufacturing to Reproduce Atherosclerotic Blood Vessels

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Physical biomodels mimicking atherosclerotic blood vessels could be an interesting tool to be applied in personalized surgical planning and even for surgeons' trainning. In this context, additive manufacturing (AM), commonly known as 3D printing, has attracted significant attention due to the potential to fabricate biomodels rapidly. However, the production of such models first requires a consensual and definitive evaluation of the mechanical properties of healthy and atherosclerotic blood vessels, to acuuratey select the adequate "printable" materials.

Keywords: atherosclerotic plaques ; mechanical properties ; standardization

1. Introduction

Constricted or occluded blood vessel segments, such as stenotic or atherosclerotic vessels, are often treated through the clinical implantation of endovascular stents [1]. Usually, such endovascular interventions are supported with image-guided procedures, as they intend to be the less invasive as possible. Minimal-invasive procedures are advantageous tools inducing fewer patient complications and faster recoveries [1]. Therefore, arterial phantoms with tunable anatomical and biomechanical properties are highly demanded experimental tools for developing and validating new instruments, imaging systems, and protocols. Moreover, phantoms generated from a patient’s medical imaging data sets can serve as personalized models for diagnostic and treatment planning purposes, enabling personalized medicine [2].

These engineered biomodels can also be utilized to study the biophysical and biomechanical phenomena in atherosclerosis development, validate numerical studies, and complement or confirm results from in vivo experiments [3]. Engineered vascular biomodels can overcome limitations related to *in vivo* experimentation, such as expensiveness, low reproducibility, and ethical issues [3]. However, these phantoms must be easily reproducible and have accurately specified geometries to achieve precise training performance and reliable experimental results. This can be effortlessly accomplished with AM.

Initially developed in the 1980s, additive manufacturing, commonly known as “3D printing,” has become widely used in different areas, including medicine and healthcare [4]. Its use allows manufacturing of 3D objects as the result of a consecutive combination of 2D layers, which are slices from a digital file representing the object. As Guarnera et al. (2021) [4] wrote, “this layer-by-layer creation from a digital file enables creating complex geometries from various materials, including thermoplastic, metal, elastomers, and biomaterials”. The main advantage of 3D printing is obtaining custom-made models at a relatively low cost, with no expensive molds or casts required [5]. This is particularly beneficial to physicians as they can use those models (derived from 3D reconstructed images) for surgical planning, education, and training [6].

According to ASTM/ISO 52900:2021, AM can be categorized into seven processes [7]. The most used processes for polymeric materials are material jetting (e.g., polyjet technology), material extrusion (e.g., fused filament fabrication—FFF), vat photopolymerization (e.g., stereolithography—SLA), and powder bed fusion (e.g., selective laser sintering—SLS) [5]. The choice of adequate technology relies on the engineering requirements of the final product, namely its resolution, material properties, and cost. For instance, when printing biomimetic parts or surgical objects, the accuracy of the geometries is a highly relevant factor [4].

SLA is the most frequently used technology where a photosensitive polymer resin or hydrogel is polymerized due to the irradiation with suitable wavelengths, often in the UV range, they crosslink, giving rise to a thermosetting polymer layer-by-layer (additively). Although this strategy allows high resolution and relatively quick printing, the need for photosensitivity monomers limits the range of materials used in SLA. Additionally, thermal processing of the photosensitive material is

often required, being a barrier to low-cost printing. However, SLA is still the easiest method to print surgical models quickly and accurately [5].

Alternatively, in SLS, a powder solidifies into layers upon the irradiation with a CO_2 laser. Over time, the repeated layers of solidified powder will originate the desired object. Since large amounts of materials can be used in SLS, this 3D printing technology is very attractive in specific circumstances. However, despite having high accuracy and resolution, the porosity of resulted objects is generally undesired, making it an expensive and time-consuming technique because of the required post-treatment [4].

On the other hand, FFF technology does not involve any irradiation process or binding substance; instead, it consists of a filament extruded and deposited by a nozzle onto the printer's base. This AM technology also minimizes waste material and reduces the associated cost because instead of solidifying the material (e.g., resin or powder) in a base or tray, FFF will just utilize the needed material by depositing it. Nevertheless, the materials must be "printable", meaning that only certain materials can be deposited; nevertheless, it is the technology with the more extensive library of thermoplastic polymers available [5].

Finally, the polyjet uses an inkjet print head that jets photopolymerizable polymer droplets, which are then cured with UV radiation. As Guarnera et al. (2021) [4] wrote, since "multiple photopolymers can be deposited prior to the UV exposure, polyjet is a rare example of a commercial printer able to manufacture multi-material parts in a single print". This means that polyjet printers can fabricate a large variety of materials with high resolution and at a low cost. Moreover, the polyjet approach enables the combination of different types of materials, combining soft rubber-like polymers with stiffer polymers and allowing printing materials more similar to biological tissues [8].

2. 3D-Printed Atherosclerotic Blood Vessels

As an auxiliary tool to the cardiovascular medical field, AM has emerged in the last decades and has already demonstrated its potential. The variety of current and developing uses for the treatment of vascular disease includes the creation of models for education and training [8][9], surgical planning [10][11], and vascular device and tissue engineering [12].

Nevertheless, the literature still needs to be expanded regarding the creation of vascular models oriented to atherosclerosis disease modeling. The shortage of work done in this field is due to the non-consensual values of the mechanical properties of such biological tissues (both health and diseased blood vessels, and atherosclerotic plaques). In fact, Ahn et al. (2018) [13] said that "it is hard to mimic atherosclerotic plaque by 3D printing technology because atherosclerotic plaque has complex composition". Even though, some research groups have already tried to reproduce atherosclerotic blood vessels by AM techniques.

In order to understand the relationship between spatial characteristics and hemodynamic variations, Yang et al. (2017) [14] performed hemodynamic analysis by computational fluid analysis in patient-specific stenotic vessel models and the SLA technique to produce a 3D printed model of those stenotic vessels. The 3D printed models were obtained from a printer with 75 µm of resolution with a smooth surface, capable of forming the precise replica of patient-specific coronary arterial models with a 1:4 scale. Models were printed with liquid resin, and the excess raw materials were manually removed. The authors found that 3D printed models not only facilitated the sensory understanding of the patient-specific left coronary arterial's spatial characteristics but also enabled a reliable visualization of the stenoses severities. Additionally, they concluded that multiple spatial characteristics could be an index of hemodynamic significance. Since 3D printed models have provided accurate replicas of the patient-specific left coronary arterial trees, they can help understand the spatial distribution of the stenosis and could be an advantage for educating and preparing medical strategies [14].

Friedrich et al. (2020) [1] have investigated the feasibility of 3D printing for vessel phantoms with user-defined stenoses made of elastic materials. Synthetic stenosis phantoms of different geometries were modeled with computer-aided design (CAD) software. Two exemplary stenosis geometries (asymmetric and symmetric stenosis) were 3D-printed (5 cm in length, 6 mm of inner diameter, and wall thickness of 0.4 mm) with a commercially available SLA printer using an "elastic resin" provided by the manufacturer of the printer, which has a Shore hardness of 50A and silicone like appearance and seems to be a good candidate for elastic vessel phantoms. While in asymmetric geometry, stenosis is in just one side of the vessel with a spherical shape that occludes 50% of the vessel's cross-sectional area, symmetric geometry features a rotationally symmetric hourglass-shaped stenosis that occludes 75% of the vessel's cross-sectional area. The authors found that the transparent appearance of the printing material allowed for visual feedback during a simulated procedure, which is an advantage for testing a clinical intervention. Additionally, as the design freedom gained by AM is large, printed

phantoms can be easily adapted to the requirements of the study, allowing to make reproducible tests with precisely patient-specific geometries [1].

On the other hand, Carvalho et al. (2020) [3], developed a hemodynamic study in idealized stenotic and healthy coronary arteries by a high-speed video microscopy technique. Experimental flow studies were performed in biomodels with three different resolutions (50, 100, and 150 µm) obtained from SLA 3D printing technology. Biomodels were designed in an online platform and custom-manufactured using a rigid material. Although it was a simplified model, the selected dimensions of the 3D printed biomodel allowed for obtaining good enough results to validate numerical results. The stenosis length (6 mm) was defined as twice the inlet diameter (3 mm), and the models' total length was defined as 50 mm. Different degrees of stenosis were considered: 0% (healthy model), 50%, 60%, 70%, and 80%, corresponding to the diameter reduction at the stenosis throat. The authors concluded that the biomodel printed with a resolution of 50 µm, due to its lowest roughness values, was able to give more accurately results and precise flow visualization. They also found good agreement between experimental flow results and blood flow numerical data [3].

After concluding the best biomodel's parameters, Carvalho et al. (2021) [15] continued their work and did a hemodynamic study in 3D printed stenotic coronary artery models to evaluate the influence of stenosis degree in blood flow distribution. The selected dimensions of the 3D printed biomodel were the same as those used in previous work [3] (length of stenosis of 6 mm, inlet diameter of 3 mm, and model's total length of 50 mm). Different degrees of stenosis were also considered: 0% (healthy model), 50%, 60%, 70%, and 80%, corresponding to the diameter reduction at the stenosis throat. The 3D biomodels were designed in an online platform and manufactured by using an SLA printer, with a printing resolution of 50 µm (the material was not specified). Besides validating numerical calculations with 3D printed biomodels, Carvalho and co-workers have also concluded that stenosis degrees higher than 50% create disturbed flow downstream of the contraction [15]. Researchers provided a pathophysiologic study about atherosclerosis's effect on blood vessels' hemodynamic performance without using in vivo experiments (which are expensive, time-consuming, and have ethical issues) but with accurate results that can be transposed to human physiology.

To produce a promising platform to elucidate the pathophysiology of atherosclerosis and seek effective drugs and therapies, Gao et al. (2021) [16] constructed an atherosclerotic model via a novel fabrication strategy. Atherosclerotic biomodels were developed using a 3D in-bath coaxial cell printing technique resulting in a triple-layered artery equivalent model with tunable geometries. The fabrication process involved the 3D printing of a model house, deposition of bath material, coaxial cell printing of dual-layered tubes, and pump connection. The study reconstructed a native atherosclerotic environment involving co-culture cells and local flow signaling [16].

On the other hand, Guarnera et al. (2021) [4] developed a mimicking model of the external iliac artery affected by atherosclerotic plaque, employing the polyjet multi-material technique to be mechanically tested and to validate different numerical models. Polyjet print technology was chosen for its ability to print multiple materials within a single part at the required dimensions (10 mm × 40 mm × 10 mm) and resolution. In fact, 3D printed model contained six regions with distinct mechanical responses. To accomplish that, five digital materials (materials that result from the combination of soft rubber-like polymers with stiffer polymers) were produced by polyjet printing. The digital materials are mixtures obtained through jetting of two different materials: high flexible photopolymer with the ability to undergo large deformations (Agilus30) and a rigid photopolymer (VeroClear®). As the percentage of VeroClear® increases, the hardness of the material also increases. The geometry of the 3D printed biomodel was based on the cross-section of the human external iliac artery affected by atherosclerotic plaque obtained from a high-resolution magnetic resonance image discretized through the Carrera Unified Formulation model according to mechanical properties of atherosclerotic components. To mimic adventitia, calcification deposit, fibrous cap, fibrotic media, media, and lipid pool, the authors utilized digital materials with Shore A hardness values of 50A, 95A, 50A, 70A, 40A, and 30A, respectively. Different Shore A hardness values are obtained by varying the composition of the digital materials. Mechanical characterization of each type of digital material was performed through quasi-static tensile testing, and authors found that the stiffness values of the materials were three times greater than those estimated in biological tissues [4]. Although this study was a scientific advance in atherosclerotic plaque modeling, additional work must be done to produce a more accurate biomodel of an atherosclerotic blood vessel in biomechanical properties. Future biomodels must have discretized regions, each one corresponding to a different plaque component and with mechanical properties similar to the biological tissues that are mimicking.

More recently, Song et al. (2022) [17] created an experimental model of real-structure carotid arteries with stenosis caused by atherosclerotic plaques, applying 3D printing technology. Researchers aimed to obtain a 3D printed model based on real configuration data to simulate the stenotic blood flow caused by carotid plaques. Flow field characteristics of carotid artery stenosis were revealed through full velocity field measurements. Then, numerical simulation calculations were done to confirm and validate physical experimental data. The physical experimental model was converted from a 3D geometric

model of carotid arteries with plaques reconstructed from computed tomography (CT) images. The 3D printed model had a layer thickness of 0.1 mm and was achieved by the SLA technique using a transparent photosensitive resin. The authors concluded that 3D printed models could support the understanding of carotid artery stenosis flow characteristics accurately, and that numerical simulation was a reliable method for studying the blood flow under stenotic conditions [17].

Table 1 summarizes the most used materials in 3D printing of blood vessels, although most of the works do not specify the chemistry of the polymeric material. Moreover, some critical reviews on 3D printing and polymers to reproduce biological tissues environments can also be consulted, such as those of references [18][19].

Table 1. Materials used in additive manufacturing to reproduce vasculatures.

Material	AM Technology	Processing Methods	Observations	Is Atherosclerosis Approached?	Ref
Liquid resin	**SLA**	**Printer resolution of 75 µm. Printed models with smooth surface. Raw material manually removed.**	**Better understanding about the relationship between spatial characteristics of stenotic arteries and their hemodynamic performance.**	**Yes**	[14]
Elastic resin with silicone like appearance (provided by manufacturer)	**SLA**	**3D printed phantoms with 0.4 mm of wall thickness.**	**Transparency of phantoms allows visual feedback during surgical protocol training.**	**Yes**	[1]
Rigid material	**SLA**	**Biomodels printed with three different resolutions and five different stenotic degrees.**	**Biomodels printed with better resolutions (50 µm) allow reliable results.**	**Yes**	[3]
NA	**SLA**	**Printing resolution of 50 µm. Biomodels printed with five different stenotic degrees.**	**Stenotic degrees higher than 50% disturbs the blood flow downstream the stenosis. Improved knowledge about stenosis physiology with no in vivo experimentation.**	**Yes**	[15]
Agilus30 (flexible photopolymer) and VeroClear® (rigid photopolymer)	**Polyjet**	**3D printed models with six different regions, representing different mechanical responses.**	**Differences between the mechanical properties of models and mechanical properties of corresponding biological tissues are very large.**	**Yes**	[4]
Transparent photosensitive resin	**SLA**	**Biomodels printed with a layer thickness of 0.1 mm.**	**3D printed models allow better understanding about the blood flow under stenotic conditions.**	**Yes**	[17]

NA—not available.

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