3D and 4D Printing of Biomedical Materials (BMMs)

A.A. Gayan Kavinda Siriwardana

Department of Materials and Mechanical Technology, Faculty of Technology,University of Sri Jayewardenepura

Introduction

Three-dimensional (3D) printing, or additive manufacturing (AM), is a novel technology that fabricates materials on a print bed layer-by-layer. AM manufactures objects with simple to complex geometries using computer-aided design (CAD) models. AM can process various materials, such as polymers, hydrogels, ceramics, glass, metals, and other composites. Several AM-based techniques with different material processing technologies, including material extrusion, vat photopolymerization (VP), powder bed fusion (PBF), material jetting (MJ), binder jetting (BJ), directed energy deposition (DED), and sheet lamination are currently in use. The main AM techniques currently active in manufacturing biomedical materials (BMMs) are fused deposition modeling (FDM), stereolithography (SLA), selective laser sintering (SLS), PBF, DED, BJ, and bioprinting.

Four-dimensional (4D) printing is considered the next-generation advancement of AM technology, adding a fourth dimension as the time-dependent shape/functional change after printing. 4D printing processes smart materials capable of changing the shape or function upon exposure to certain stimuli such as humidity, temperature, light, pH of the medium, solvent, and magnetic and electric fields. Shape memory polymers (SMPs) play a key role in this context. AM technologies involved in 4D printed BMMs are mainly direct ink write (DIW), SLA, and multi-MJ, targeting applications in tissue engineering, drug delivery, medical devices, and diagnostics.

BMMs are broadly defined as biomaterials manufactured or processed to be utilized as medical devices or related components. BMMs include prostheses, reconstituted tissues, intravenous catheters, sutures, implants (prosthetic heart valves, ureteral stents, and hernia meshes), and scaffolds [15]. Currently, AM contributes to the manufacturing of BMMs mainly *via* polymers, ceramics, bioactive glass (BG), metals/alloys, and composites for biomedical applications such as tissue engineering, drug delivery, porous metal implants, cell-materials interactions, wear degradation, bionanotechnology, and biopharmaceuticals.

Tissue Engineering

3D Printing

Tissue engineering, a discipline of biomedical engineering, uses a combination of cells, engineered materials and methods, and suitable biochemical and physiochemical factors to restore, maintain, improve, or replace various types of biological tissues. The primary criteria for a polymer to be qualified for tissue engineering applications are its high bioresorbability or biodegradability, high mechanical strength, and enhanced cell attachment ability. Recently, Kim *et al.* 2021 prepared a bioink by combining alginate (Alg) and silk fibroin (SF) protein to fabricate hydrogel scaffolds using DIW and visible light irradiation (**Figure 1A**). SLM-based 3D printed hydrogel scaffolds containing platelet-rich plasma (PRP)- gelatin methacrylate (GelMA) have also been studied. While BJ and SLA have frequently been used to process bioceramics, various AM techniques, such as FDM, DIW, DED, and SLS, have been employed. Ceramic-polymer composites have also been successfully 3D printed using polymers like polycaprolactone (PCL), PLA, or polylactide glycolic acid (PLGA) with calcium phosphate (CaP). Bose *et al.* 2021 demonstrated successful 3D printing of scaffolds *via* BJ. 45S5 bioglass (BG)-based scaffolds have also been 3D printed *via* the SLS AM technique and DIW.

4D Printing

Gugulothu and Chatterjee,2023 demonstrated a successful 4D printed bioink consisting of a blend of GelMA and poly(ethylene glycol) dimethacrylate (PEGDM) with a photoinitiator and a photoabsorber *via* DLP technique. This 4D bioprinted material acts as a shape morphing and cell-laden hydrogel for tissue engineering applications by supporting cell viability and proliferation while altering its shape upon hydration, a cell-friendly stimulus (**Figure 1B and C**). Using alginate dialdehyde (ADA) and Gel, Kitana *et al.* 2023 exhibited DIW-based 3D printed tubular structures that self-transformed into a T-junction after immersing in water, hence showing 4D printing behaviour. The transformation of the 4D printed crosslinked ADA/Gel, into a tubular T-junction after swelling in pure water is depicted in (**Figure 1D**).

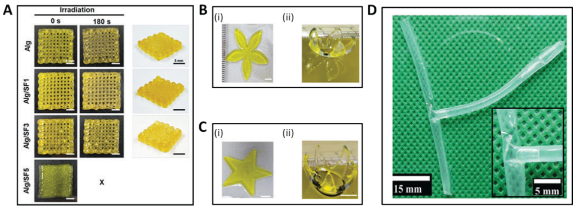

Figure 1. Recently developed 3D and 4D printed BMMs for tissue engineering. (A) Adapted from Kim *et al.* 2021. (B) and (C) Adapted from Gugulothu and Chatterjee,2023. (D) Adapted from Kitana *et al.* 2023.

Drug Delivery

3D Printing

Drug delivery refers to a broader scientific field involving various approaches, formulations, manufacturing techniques, storage systems, and technologies that transport a pharmaceutical compound to a specific target site to obtain a desired therapeutic effect. Polymers such as PLGA, PCL, and other materials like CaP ceramics, BGs, bioactive ceramics, and ceramic-polymer pastes have been explored in this field. The BJ AM technique enables the delivery of heat-labile molecules such as growth factors and antibiotics by fabricating low-temperature CaP-based scaffolds. Martínez-Vázquez *et al.* 2015 described successful DIW-based 3D printing of porous silicon-doped hydroxyapatite (HASi) and Gel composite scaffolds for delivering vancomycin antibiotics. Koski *et al.* 2018 demonstrated the use of naturally sourced gelatinized starch as a natural binder system with HA ceramic to obtain extrusion-based solid-freeform fabricator (SFF) scaffolds. These scaffolds showed improved compressive strength and in vitro biocompatibility with osteoblast cells without crosslinking or post-processing. Liang *et al.* 2018 reported PLA/ polyvinyl alcohol (PVA)-based FDM-3D printed mouthguard (**Figure 2A–D**) loaded with food grade flavor vanillic acid (VA) and clobetasol propionate (CBS) model drug.

4D Printing

4D printing is applied in numerous advanced drug delivery systems to improve efficiency in treatment outcomes. Controlled release of drugs, patient-specific dosing, and targeted delivery are the main advantages of these 4D printed structures compared to conventional systems. Fang *et al.* 2020 published polymer/carbon-based magnetoelectric responsive porous nanocookie conduit 4D printed *via* DLP (**Figure 2E**) while Mohammed *et al.* 2021 demonstrated ethyl cellulose/ hydroxypropyl methylcellulose (HPMC)/polyvinyl pyrrolidone (PVP)/cellulose acetate-based controlled drug release shell (**Figure 2F and G**) 3D printed *via* pressure-assisted microsyringes (PAM) technology.

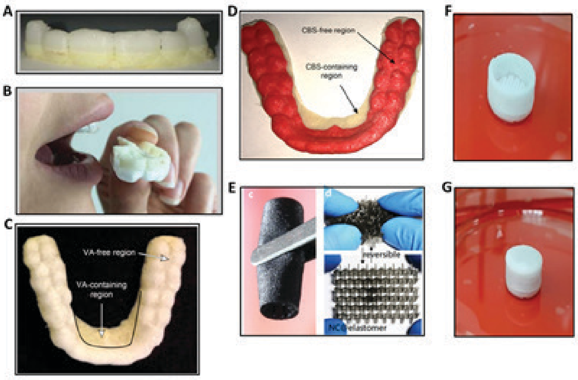

Figure 2. Recent 3D and 4D printing advancements in drug delivery. (A-D) Adapted from Liang *et al.* 2018. (E) Adapted from Fang *et al.* 2020. (F-G) Adapted from Mohammed *et al.* 2021.

Surgical and Diagnostic Tools

3D Printing

A surgical tool or instrument is a medical device for performing specific actions or carrying out desired effects during surgery, including the modification of biological tissues. Surgical tools can be prepared with AM techniques such as MJ with thermoplastics and thermosets and BJ and PBF-based techniques like SLS and SLM with metals, ceramics, polymers, and glasses. George *et al.* 2017 developed an SLS-based AM of a surgical tool kit, including hemostats, needle drivers, scalpel handles, retractors, and forceps, using virgin and recycled Dura-Form EX plastic powder. In another study, Wu and co-workers, 2019 displayed an FDM-3D printed silver nanoparticle-polyacrylamide (AgNP-Pam)/HPMC-based superporous hydrogel for wound dressing applications (**Figure 3A**).

Medical diagnosis determines which disease or condition is responsible for a set of symptoms/signs. The devices that are utilized in this detection process are referred to as diagnostic tools, which include equipment such as stethoscope, blood pressure monitors, pulse oximeters, electrocardiographs (ECGs), electroencephalography (EEGs), ultrasonography (US), X-ray machines, and biosensors. Recent literature illustrates medical diagnostic tools fabricated *via* FDM, DIW, SLA, SLM, and SLS-based AM techniques. Ren and co-workers, 2019 3D printed a thermoplastic frame or a device using FDM to support a magnetic focus lateral flow sensor (**Figure 3B**) detecting and diagnosing cervical cancer biomarkers. Kuo *et al.* 2019 developed a microfluidic device *via* SLA using low molecular weight poly(ethylene glycol) diacrylate (PEGDA) at sub-millimeter resolution. They fabricated an active micro-mixer containing pneumatic micro-valves and micro-channels with high resolution. Studies by Vandenbroucke and co-workers, 2007 showed that biocompatible metal alloys, Ti-6Al-4V and cobalt-chromium-molybdenum (Co-Cr-Mo), yield SLM-based 3D printed parts used as dental prostheses. These parts met the strength, stiffness, corrosion behaviour, and process precision standards for medical and dental applications.

4D Printing

4D printing technology can fabricate smart surgical tools adaptable to environmental changes. These are functionally tailored to provide precision and control during complex surgical procedures. Using high-resolution projection microstereolithography (PμSL) and multiple shape memory polymers, Ge and co-workers, 2016 successfully 4D printed a surgical gripper system that changed its shape and stiffness responding to stimuli within the human body (**Figure 3C**). These properties could enable this gripper system to steer through tight spaces, grasp, and control fragile tissues while minimizing the risk of damage during surgical work.

4D printing has recently demonstrated immense potential in developing advanced diagnostic tools for the medical field. Owing to the ability to respond to external stimuli, 4D printed diagnostic tools offer more sensitivity, accuracy, and patient-specificity, leading to improved patient care. Kumar *et al.* 2023 introduced a wearable smart sensor made from thermoplastic polyurethane (TPU) 4D printed on fabric *via* FDM. Colvill and co-workers, 2020 succeeded in 4D printing a deformable lung, including respiratory tract and liver phantom. This helps assess the accuracy of computed tomography (CT) and magnetic resonance (MR) imaging in radiotherapy planning.

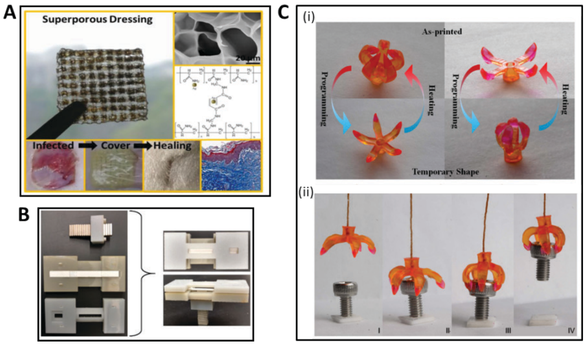

Figure 3. Recent contributions in 3D and 4D printing towards surgical/diagnostic tools. (A) Adapted from Wu and co-workers, 2019. (B) Adapted from Ren and co-workers, 2019. (C) Adapted from Ge and co-workers, 2016.

Implants and Prosthetics

3D Printing

Medical implants are devices placed in or on the body surface. The most common implants are prosthetics intended to replace a damaged or missing body part. Apart from prosthetics, other implants deliver medicines to internal organs and tissues, support internal structures and monitor body functions. Implants can be made from biological materials such as bones, tissues, skin, metal, plastic, ceramic, and other composite materials. Chakraborty *et al* 2023 recently reported Ti-6Al-4V based porous channel dental implants 3D printed *via* DMLS (**Figure 4A**). In another recent study, Moiduddin *et al.* 2023 published FDM-3D printed acrylonitrile butadiene styrene (ABS)-based human skull and PEEK based porous implant applied on the skull (**Figure 4B**). Tappa *et al.* 2019 demonstrated

FDM-3D printed PLA/antibiotic based interference fixation screws (**Figure 4C**).

4D Printing

4D printing technology has also contributed remarkably to fabricating smart implants and prosthetics. Its ability to respond to specific stimuli, such as temperature, moisture, light, and magnetic fields, has yielded customizable and adaptable implants and prosthetics. Wei *et al.* 2017 evidenced successfully cross-linked PLA-based thermomagnetic responsive vascular stent 4D printed *via* DIW (Figure 4D) while Kuang *et al.* 2018 reported PCL/acrylates-based thermo responsive vascular conduit 4D printed *via* DIW (**Figure 4E**).

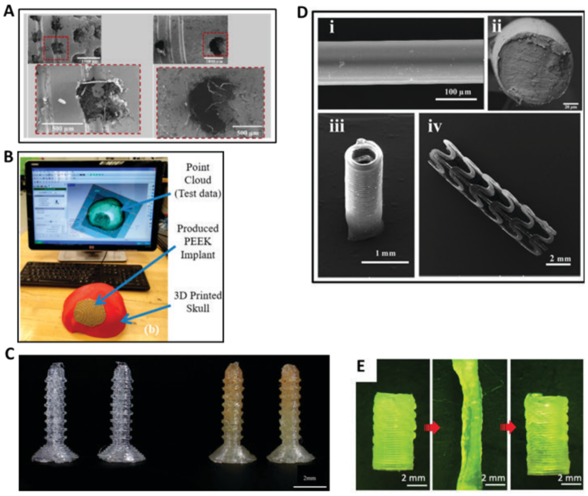

Figure 4. 3D and 4D printed BMMs for implants and prosthetics. (A) Adapted from Chakraborty *et al* 2023. (B) Adapted from Moiduddin *et al.* 2023. (C) Adapted from Tappa *et al.* 2019. (D) Adapted from Wei *et al.* 2017. (E) Adapted from Kuang *et al.* 2018.

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***Dr. A. A. Gayan K. Siriwardana** obtained his B.Sc (Hons), University of Kelaniya, M.Sc. and PhD in Chemistry at Sam Houston State University, TX, USA. At present, he serves as a Senior Lecturer, Department of Materials and Mechanical Technology, Faculty of Technology, University of Sri Jayewardenepura.*

Guest Articles

Engineering of Non-Ribosomal Peptide Synthetases: Advances and Applications

Rajendran Sanjeevan

Department of Chemistry, BioDiscovery Institute, University of North Texas, Denton, Texas, USA

1. Introduction

Natural products are small molecules synthesized by fungi, bacteria and plants, which historically have had a profound effect on human health and quality of life. Natural products are classified according to their biosynthetic origin exemplified by polyketides, non-ribosomal peptides (Figure 1), hybrid molecules and terpenes. Non-ribosomal peptide synthetases (NRPSs) are complex and versatile enzymes responsible for the biosynthesis of a diverse range of peptides, which include many bioactive compounds. Unlike ribosomal peptide synthesis, NRPSs operate through a modular system that does not rely on mRNA templates, allowing for the production of peptides with complex structures and functions. The concept of combinatorial biosynthesis[1] involves expanding the biosynthetic inventory of a fungal producer by introducing non-native enzymes into specific biosynthetic pathways, ultimately manipulating the natural product output. Engineering NRPSs offers significant potential for creating novel peptides with applications spanning pharmaceuticals, agriculture, and biotechnology. This article discusses the principles of NRPS engineering, recent advancements, and future prospects.

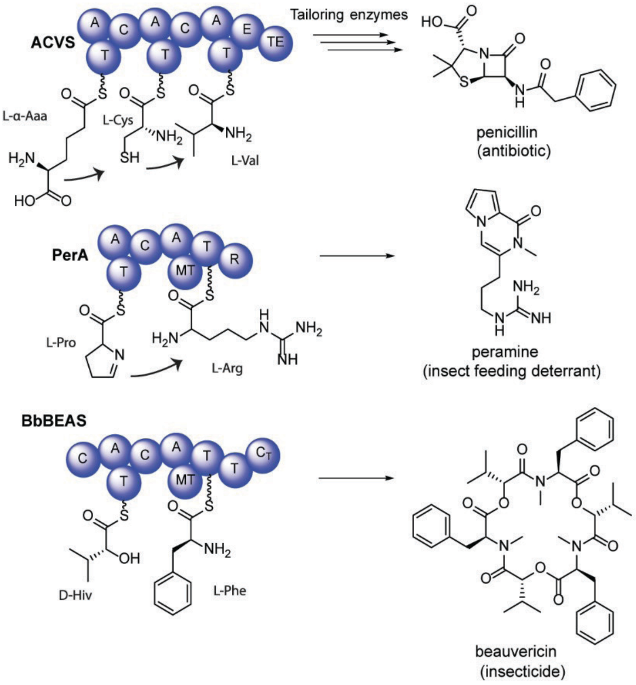

Figure 1. Examples of fungal natural products and the module / domain composition of each megasynth(et)ase that produces them. Common building blocks of amino acids supply precursors for the biosynthesis of non-ribosomal peptides. Domain abbreviations: MT = methyltransferase; TE = thiolesterase; R = reductase; C = condensation; A = adenylation; T = thiolation (also referred to as PCP = peptidyl carrier protein); E = epimerase.2