Recent Advances in 3D Printing of Polyhydroxyalkanoates: A Review

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Abstract

In the 21st century, additive manufacturing technologies have gained in popularity mainly due to benefits such as rapid prototyping, faster small production runs, flexibility and space for innovations, non-complexity of the process and broad affordability. In order to meet diverse requirements that 3D models have to meet, it is necessary to develop new 3D printing technologies as well as processed materials. This review is focused on 3D printing technologies applicable for polyhydroxyalkanoates (PHAs). PHAs are thermoplastics regarded as a green alternative to petrochemical polymers. The 3D printing technologies presented as available for PHAs are selective laser sintering and fused deposition modeling. Stereolithography can also be applied provided that the molecular weight and functional end groups of the PHA are adjusted for photopolymerization. The chemical and physical properties primarily influence the processing of PHAs by 3D printing technologies. The intensive research for the fabrication of 3D objects based on PHA has been applied to fulfil criteria of rapid and customized prototyping mainly in the medical area.

Keywords: 3D printing, polyhydroxyalkanoates, biopolymers, stereolithography, fused deposition modeling, selective laser sintering, bioprinting

Introduction

Polyhydroxyalkanoates (PHA) are thermoplastic polyesters of hydroxyalkanoic acids biotechnologically produced by fermentation of numerous bacteria and extremophilic archaea (1). These microorganisms are cultivated in special fermentation media, which contain sources of carbon, nitrogen, phosphorous and minerals. The great benefit for the environment and targeted waste management is that carbon sources for biotechnological production of PHA can be derived from industrial waste substrates such as soya and malt waste (2), waste oil (3), spent coffee grounds (4), winery waste (5) and many others. PHAs are formed in accumulating microorganisms as an intracellular secondary product in the form of water-insoluble granules and are stored in the cytoplasm. The deposition of PHA granules occurs in the stationary phase of the growth curve of the microorganism under conditions of the carbons source excess and a lack of the nitrogen source in the fermentation medium.

So far, over 150 monomers were identified in structures of biosynthesized PHAs. The variability of PHA composition is broad and depends mainly on the production microorganism and the composition of fermentation media. A typical producer of poly(3-hydroxybutyrate) (P3HB) is *Cupriavidus necator*. However, if a suitable precursor is supplemented during the synthesis, *C. necator* can synthesize poly(3-hydroxy-co-3-hydroxyvalerate) (P3HBMV) (6, 7). The composition of the polymer as well as conditions used in the upstream and downstream processes, affect its molecular weight, polydispersity, degree of crystallinity and purity (8). Theoretically, PHAs are divided according to the length of the polymer chain into three groups: polymers with short-chain length (3-5 carbons, scl-PHA), polymers with medium-chain length (6-14 carbons, mcl-PHA) and polymers with long-chain length (more than 14 carbons, lcl-PHA). Nevertheless, prac-

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tically only scl-PHA and mcl-PHA are available on the global polymer market. The leading companies engaged in PHA production are shown in Table 1.

Between the often produced and investigated polymer and

taken as a property of a given polymer resin with possible content of various additives because companies do not state the exact composition.

Some of the PHA manufactures offer resins from the

Table 1. List of global manufactures of PHA

Company	Headquarters	Product	Composition
Biomatera	Toronto, Canada	Biomatera	Not specified PHA
Biomer	Schwalbach, Germany	Biomer	P3HB
Bio-on	Bologna, Italy	MINERV-PHA	Not specified PHA
DaniMer Scientific	Bainbridge, Georgia, USA	Nodax PHA	Not specified PHA, e.g. P3H-B-co-3HHx
Kaneka Corporation	Tokyo, Japan	Kaneka PHBH TM	P3HB-co-3HHx
Mango Materials	Redwood City, California, USA	YOPP PHA	Not specified PHA
Nafigate	Prague, Czech Republic	Hydal	P3HB
NaturePlast	Arago, France	PHA	Not specified PHA
Newlight Technologies	Irvine, CA, USA	AirCarbon TM	Not specified PHA
RWDC Industries	Singapore, Singapore	Solon	Not specified PHA
Shenzhen Ecomann Biotechnology	Shandong, China	AmBio	Not specified PHA
SIRIM	Selangor, Malaysia	PHA	Not specified PHA
TAIF Group	Tatarstan, Russian Federation	PHA	Not specified PHA
TEPHA	Massachusetts, USA	Tephaflex, TephElas	Not specified PHA
TianAn Biological Materials	Zhejiang, China	ENMAT	P3HB and P3HBV
Tianjin Green-Bio	Taijin, China	Sogreen	Not specified PHA, e.g. P3H-B-co-4HB

P3HB – poly(3-hydroxybutyrate), P3HB-co-3HHx – poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), P3HBV – poly(3-hydroxybutyrate-co-3-hydroxyvalerate), P3HB-co-4HB – Poly(3-hydroxybutyrate-co-4-hydroxybutyrate)

copolymers belong P3HB and some of its copolymers. Thermal, rheological and mechanical properties of PHAs depend on their chemical composition and molecular weight. PHAs after biosynthesis may reach molecular weight from 0.5×10^5 to $35 \times 10^5 \text{ g mol}^{-1}$ and polydispersity from 1.1 to 6.0 (9). Physical properties of P3HB are similar to polypropylene but P3HB is more rigid and less flexible material. Its melting temperature is in the range of 170 to 180°C. The high brittleness corresponds with a high degree of crystallinity (up to 80%). The lower degree of crystallinity and higher flexibility may be reached by a formation of copolymers of 3-hydroxybutyrate with 3-hydroxyvalerate (P3HBV), 3-hydroxyhexanoate (P3HB-co-3HHx), or 4-hydroxybutyrate (P3HB-co-4HB). The semi-crystalline structure of copolymers differs from P3HB and allows the formation of materials with lower brittleness, lower stiffness and higher elongation at break. Thermal as well as mechanical properties of copolymers are variable and depend on the concentration of monomers in copolymers. Table 2 displays the thermal and mechanical properties of the selected industrial P3HB and its copolymers. It should be emphasized that this data should be

group of scl-PHA and mcl-PHA directly tailored to customer requirements. PHAs are considered as alternatives to fossil petroleum-based plastics. Their advantages are bio-origin, renewability, biodegradability, non-toxicity, biocompatibility and additional unique properties (gas barrier, UV-barrier, piezoelectricity). However, the price of approximately ten euros per kilogram of P3HB is a limiting factor for the industry. The copolymers are even more expensive due to the different synthesis strategy (e.g., the price of Enmat Y 1000P P3HBV in November 2020 was about 18 euros) (14-16). Therefore, the use of PHA in many industrial sectors is subjected to price. It makes sense to find such PHA applications where except to biocompatibility and biodegradability also other added values are given (17). Nevertheless, the current market situation shows that the demand for biopolymers began to grow gradually. The global PHA market size reached 57 million USD in 2019 and was estimated to reach 98 million USD by 2024 (18).

PHAs have been found suitable for medical applications (19, 20), food packaging (17, 21), toys, disposable tableware (22), agricultural applications (23, 24), automotive, cosmetic (25),

Table 2. Thermal and mechanical properties of the selected commercial PHAs (10-13), n – data were unlisted by the company

Polymer	Trade name	Company	Melt temperature (°C)	Tensile strength (MPa)	Elongation at break (%)	Modulus (MPa)
P3HB	Biomer P226	Biomer, Germany	180	25.5	n	1520
P3HBV	Enmat Y1000P	TianAn Biological Materials, China	170 - 176	39	2	2800 - 3500
P3HB-co-3HHx	PHBH X 151 A	Kaneka Corporation, Japan	126	26	320	950
P3HB-co-4HB	Sogreen	Tianjin Green-Bio, China	110	14	775	n

textile (26), household applications and more recently in 3D printing. Each of the applications requires different processing of the materials to meet the required properties.

3D printing technologies represent great possibilities for producing 3D models according to customer requirements. This paper reviews the possibilities of processing of PHAs by commonly used additive manufacturing technologies.

2. Additive Manufacturing technologies

3D printing is a process that allows creating three-dimensional physical objects according to designed engineering 3D models. Computer-aided design (CAD) software or other animation modelling software are used to create the 3D virtual models. Several 3D printing technologies have been developed for prototyping or manufacturing of defined objects with broad applications in a range of industrial sectors, such as education, architecture, jewellery, aerospace, building, dental, medical, pharmaceutical and automotive industry and even in the household. The basic principle of these technologies is that the object is gradually built in the form of layers of liquid, molten powder or molten filament according to a designed 3D model. The exact principles of processing and postprocessing depend on the type of polymer (thermoplastic, thermoset) and its physical form (liquid, hydrogel, powder, and filament) (27). Table 3 lists principles of commonly used additive manufacturing (AM) technologies applied for polymers.

2.1 Polyhydroxyalkanoates processed by stereolithography

Stereolithography (SL) has been used as a 3D printing method since the 1980s. The printing material is in the form of the liquid polymer that are curable through photopolymerization (e.g. UV radiation). The successively SL printing method requires photopolymerizable resins, which are cross-linked for a few seconds. The curing mechanism depends on the adopted stereolithographic strategy (polymer resins composition, source of light, light intensity, temperature). (28). As crucial factors for the unproblematic SL process are considered the effective content of photo-sensible active terminating functional groups and the viscosity lower than 5 Pa.s (29). The typical stereolithographic prepolymers are acrylate monomers, methacrylate monomers, epoxides and vinyl ethers. These materials

are non-biodegradable, insoluble materials with high density and modulus and with limited or no biocompatibility. Stereolithography is a method producing objects with outstanding accuracy and the well-finished surface. However, the standard UV curable resins are not biodegradable and usually not biocompatible and therefore not suitable for use in medicine.

Polyhydroxyalkanoates are attractive biocompatible polymers with the ability to degrade in vivo predominantly through the phagocytic ability of macrophages, foreign body giant cells and osteoclasts (30). However, PHAs do not naturally contain photo-polymerizable functional groups that would enable the curing reaction necessary for stereolithographic processing. One possible way how to develop UV-curable formulation based on PHA is to synthesize PHA-oligomers with UV curable end groups. Foli et al. presented a successful synthesis of PHA curable resins. In this two-step solvent-free synthesis was employed first the transesterification of poly(3-hydroxybutyrate) with 1,4-butanediol. In the second step, the received PHB-diol was modified by a reaction with 2-isocyanatoethyl methacrylate. Finally, PHB oligomers with methacrylate functionalities were obtained. This work showed that the developed methacrylated PHB oligomers were soluble in propylene carbonate at 90°C and after addition of photo-initiator were photo-polymerizable under UV light within 10 minutes (29).

Foli et al. presented that photocurable resins may be synthesized based on methacrylated PHB oligomers. However, it is not sure whether these materials after photopolymerization will be biocompatible and biodegradable. This should be answered through further research.

2.2 Polyhydroxyalkanoates processed by selective laser sintering

Selective laser sintering (SLS) is an AM technique based on the powder bed fusion and formation of a 3D model by the successive layerwise selective sintering by employing a CO₂ laser. This technique may serve prototyping as well as large-scale industrial production. SLS has been invented and patented by Deckard in the mid-1980s (31). Typical materials fabricated by SLS are nylon, polycarbonate, nylon/glass composite, wax, ceramics, elastomers and metals in the form of powders. Mechanical properties of produced objects are directly influenced by properties of fused materials, laser sintering processing

Table 3. Additive manufacturing technologies used for processing of polymers

3D printing technology	Principle	Polymer	Physical form
Stereolithography (SL)	Photopolymerization (radiation curing reaction of polymer in successive layers)	Liquid photosensitive polymer	Liquid
Selective laser sintering (SLS)	Steered powder bed fusion by CO ₂ laser beam (deposition of layer by layer sequence meshes, sintering and solidification)	Thermoplastics	Powder
Fused deposition modeling (FDM)	Extrusion of the molten filament (gradual deposition of layers and solidification)	Thermoplastics	Filament
3D Bioprinting	Extrusion of the molten (or dissolved) polymer and deposition of layers through syringes or cartridges and consequent solidification	Biomaterials with required viscosity and flowability	Granules, paste or gel materials
Material jetting (MJ)	The charged droplets are steered released by the nozzle on the substrate (solidification depends on the type of polymer compound, e.g., under UV light)	Various polymer solutions with the required viscosity (photopolymer or thermoplastics)	Ink

parameters (laser parameters, scan parameters and build parameters including layer thickness and build temperature) and post-processing process (32).

SLS has been announced as a suitable technique for fabrication of tissue engineering scaffolds due to the computed feasible fabrication of 3D objects composed of inorganic materials and biopolymers with designed porosity (33). Duan and Wang have employed calcium phosphate (Ca-P) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate (P3HBV) in the form of microspheres and confirmed that SLS method was successfully used for the formation of 3D scaffolds with controllable interconnecting porous structure (34). These authors further presented that Ca-P/P3HBV scaffolds prepared via SLS could be served as protective carriers for biomolecules or eventually for releasing of drugs (35). SLS scaffolds can be modified with biomolecules before 3D printing or by post-printing functionalization by adsorption (36). It was found that the porosity and mechanical properties of scaffolds could be modified by laser energy density during the selective laser sintering (37). The significant advantage of SLS technique is that PHAs despite their low thermal stability in the melt are stable during the SLS process and do not change their thermal properties and chemical composition. It means that the rest material after SLS can be reused for additional printing without the loss of the initial material properties (38).

2.3 Polyhydroxyalkanoates processed by fused deposition modeling

Fused deposition modeling (FDM) is an AM technology for the formation of 3D objects from thermoplastic filament materials by using melt extrusion and formation of the product by layer-

er-by-layer technique. This technology is also known as fused filament fabrication (FFF). The principle of this technique is the melting of the filament in a small temperature-controlled extruder and the deposition of molten polymer onto a platform with ascending order in layers (39). First, the filaments are produced by extrusion with a conventional single-screw or twin-screw extruder, depending on the composition of the extruded material. The usual requirements for the filament are low cost, good mechanical properties (sufficient stiffness, tensile strength and flexibility), good dimensional stability and good shelf life. The filaments are usually made with a diameter of 1.75 mm. Filament market offers filaments made of acrylonitrile butadiene styrene (ABS), acrylonitrile styrene acrylate (ASA), polyamide (PA), poly(lactic acid) (PLA), poly(lactic acid)/polyhydroxyalkanoates blends (PLA/PHA), polyethylene terephthalate (PET), thermoplastic copolyester (TPC) and thermoplastic elastomer (TPE). Each of these materials have their advantages and disadvantages and their choice depends on the requirements for the product. Filament manufactures offer filaments with special properties (colour, surface effect, abrasion resistance, water resistance, functionality, flexibility and printing temperature) (40).

PLA and PLA/PHA filaments are biodegradable and their products are not recommended for outdoor applications. Their most significant advantages are biodegradability and biocompatibility thus extending their use to medicine. Neat PHAs are not yet available on the market. The reason is not only their high price but also their low thermal stability during melt processing (41). The most cost-effective polymer from the PHAs is P3HB. However, P3HB undergoes significant degradation during melt processing, as is evidenced by the colour change

from white to brown and a significant decrease in molecular weight, which adversely increases its brittleness. The viscosity of P3HB without additives and modification may decrease so rapidly during melt processing in the extruder that the polymer flows like water and after solidification it acquires enormous brittleness.

The rheological properties and thermal stability of P3HB do not allow preparing a filament only from P3HB without other additives (42). Thermally more stable than P3HB is its copolymer with 3-hydroxyvalerate (3HV), P3HBV (43). Rheological and mechanical properties of P3HBV depend on the 3HV content in copolymers. As the 3HV content increases, the tensile elongation values increase but the tensile strength of the copolymer decreases (44). Some authors have tried to increase the tensile strength of P3HBV by reinforcing with filler. However, there is a risk of low interfacial compatibility between P3HBV and filler, which impairs the mechanical properties of the composite. The production of PHA composites requires the chemical modification of the polymer matrix and/or filler (45, 46). Wu and his colleagues presented the successful preparation of filaments and 3D objects processed by FDM on the base of esterified P3HBV and different filler, such as palm fibres, carbon nanotubes and siliceous sponge spicules. Esterification of P3HBV has proven to be an effective method for improving polymer/filler adhesion without adversely affecting the desired material properties obtained by the addition of filler (mechanical, thermal, conductive, antistatic or antibacterial properties) (47-49). Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate (P3HB-co-3HHx) is another copolymer that can be extruded to produce filaments and subsequently processed by FDM. Its rheological properties and thermal stability during melt processing are comparable with PLA (42, 50).

The exact composition of PLA/PHA filaments offered on the market is not known; however, the most preferred combination is PLA with P3HB or P3HBV (51). For the advanced modification of rheological and mechanical properties of PLA/PHA blends can be added plasticizers (52, 53). It was found that the blending of PLA with PHA up to 20% can markedly improve the final ductility of filament. The reason is the formation of the uniformly distributed PHA phase in the PLA matrix. The final modified crystalline morphology suppresses the brittle failure of neat PLA and improves the impact strength (54, 55). Mechanical properties of 3D objects mainly depend on the polymer and used additives (56), and the applied filling texture. As the filling texture, which may give to the 3D object high compression strength and bending strength, was selected the diamond-celled core topology (57). Furthermore, set FDM parameters and post-processing period also contribute to the mechanical properties of 3D objects (58-60). The semi-crystalline polymer undergoes different phase changes during melting, solidification, crystallization and heat changes along with the post-processing treatment. These phase processes may cause shrinkage which can lead to mechanical defects (61). Therefore, during the planning phase of FDM of 3D objects must be thoroughly selected the following parameters, such as the

character of filament (polymer type, thermal and rheological properties of filament), printing conditions (an internal and external specimen architecture, printing temperature, working platform temperature, printing orientation, layer height) and post-processing conditions.

2.3.1 3D Bioprinting

The 3D bioprinting is AM methodology for the printing of 3D scaffolds using biopolymers in the physical form of granules, gel or paste. The material with the low viscosity can be: 1) directly extruded from a cartridge (a device for melting of granules) or 2) pressed through a syringe (a device for dosing of materials in the form of gel and paste). One possibility is to use a polymer solution which is coagulated in a targeted manner in another solution (62).

Both devices, the extruder and syringe precisely dispense materials on the building platform. Rheological properties of layers are transformed by ambient conditions to the stage permitting to apply layer-to-layer build system. The bioplotting gives the possibility to adjust the defined outer and inner structure of scaffolds, which facilitates to create tissues and organs according to particular requirements. This methodology is popular in the medical research for the fabrication of scaffolds based on different medical polymers, such as polylactides (63), gelatin ethacryloyl/polyethylene(glycol)diacrylate/alginate (64, 65), poly(ϵ -caprolactone) (66), gelatin/alginate, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/poly(ϵ -caprolactone) (62), poly(lactic acid)/poly(3-hydroxybutyrate) (67) and many others (68). The advantage is the possible combination of multiple materials in the same printing process. PHAs can be processed by the bioprinting method in the form of a solution, consequently precipitated into ethanol or directly by extrusion of a melted polymer. In the case of melt processing, polymer granules should be properly dried before using and the heating time should be kept to a minimum. In another case, there is a risk of material degradation due to the narrow processing temperature window and high sensitivity to hydrolysis (67).

Conclusions

Polyhydroxyalkanoates are biopolymers offering a whole range of exciting properties that are unique compared to common petroleum-based polymers. Their application makes sense, especially in the applications where non-toxicity, biocompatibility and ability to biodegrade are required. At a time when society is burdened with large amounts of plastic waste and concerns about contamination of nature and water with plastic microparticles, PHAs are proving to be an excellent alternative to address this situation. However, it should be emphasized that PHAs possess a large group of polymers with different chemical structures and properties.

Theoretically, PHAs can be processed by conventional polymer processing methods, but with some limitations, mainly due to their narrow thermal processing window. 3D printing technologies belong to relatively new processing technologies enabling prototyping as well as the production of unique 3D

objects for various segments of the industry.

There are several additive manufacturing methods suitable for thermoplastics and thermosets. This review discusses the applicability of stereolithography, selective laser sintering and fused deposition modeling to process PHAs and fabricate biodegradable 3D products. Each of these methods work on a different processing principle and require polymers in a different physical state. The choice of AM methods depends on product requirements and applications. One of the most popular and the most widespread 3D printing method is fused deposition modeling. The fabrication of 3D models on the base of PHAs by FDM is possible but with limitation on the selection of specific copolymers, modification of material composition with additives or blending with poly(lactic acid).

The question is whether the modification of PHA composition or its mixture will not adversely affect the properties for which it has been selected for a given application and whether the final price of the 3D product still will be acceptable. The most significant use of AM technologies for PHA processing seems to be in tissue engineering and regenerative medicine.

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