DETERMINATION OF THE GEOMETRICAL AND VISCOELASTIC PROPERTIES OF SCAFFOLDS MADE BY ADDITIVE MANUFACTURING USING BIOPLOTTER

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Abstract

Additive Manufacturing (AM) is a name of a group of technologies that build 3D objects by adding layer-upon-layer of material. There are many technologies, including Rapid Prototyping (RP), Direct Digital Manufacturing (DDM), layered manufacturing and additive fabrication. Many types of materials can be used for AM technology. Biodegradable polymers such as polylactic acid (PLA) and polyhydroxybutyrate (PHB), are currently the subject of intensive research in the field of additive manufacturing and regenerative medicine. A number of biodegradable and bioresorbable materials, as well as scaffold designs, have been experimentally and clinically studied in many research facilities around the world. For effective using of bioprinting technologies in tissue and biomedical engineering, the knowledge of material and technological parameters in the process of printing is necessary. In this study the 3D printer Bioplotter EnvisionTEC (the printer with ability to print different materials from hydrogel to plastic materials) was used. Scaffolds for the purpose of the experiment were prepared via extrusion-based bioprinting. Experimental part of this study was focused on defining the influence of printing parameters and technological pre-processing of the material on quality and mechanical and geometrical properties of printed parts. Testing of printed samples showed high influence of pre-processing of material, mainly drying process, on mechanical and geometric quality of samples. Drying of material before printing process makes the material more stable and allows it to maintain defined material properties for a longer time than non-dried material. Time of heating of the material in printing cartridge has also high impact on material behaviour. Test results showed that if the time of heating of the material in the high temperature cartridge exceeds defined time limit, the material starts to degrade and is no more usable.

Keywords

Polylactic acid (PLA), Polyhydroxybutyrate (PHB), 3D printing, Bioplotter, extrusion-based printing, polymers, scaffolds

Introduction

Understanding principles of physics, electronics, mechanics, engineering of materials as well as understanding the reactions of the human body of foreign materials used in creating various types of implantable medical devices is a key factor for every researcher interested in the field of biomedical engineering. Nowadays, new types of materials and new technologies are being invented to help scientists and engineers reach their goals in all kinds of industrial or medical research. In the field of tissue engineering and regenerative medicine, it is crucial to be able to manufacture tissue mimicking structures [1]. Most common strategy of production of the biomaterial implant structure is creating cell-seeded polymeric scaffolds [2]. However, scaffold manufacturing technologies face a challenge in achieving the ability to precisely control the parameters of three-dimensional printing of scaffold structure with the incorporation of cells during the fabrication process [2, 3].

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The conventional techniques for scaffold fabrication include fiber bonding, solvent casting, particulate leaching, membrane lamination and melt molding.

The field of additive manufacturing has great potential for creating replacements of hard tissues such as bones and cartilage, or soft tissues, such as vascular and skin grafts. These applications represent the greatest potential for tissue engineering and regenerative medicine.

3D bioprinting is the process of automated deposition of biological molecules on a substrate to form a 3D heterogeneous functional structure with data derived from a digital model [4].

The basic aspect which affects the process of printing is the right selection of materials. The material for 3D printing using bioprinting techniques, also known as bio-ink, often includes various combinations of biological living cells, polymers, chemical factors and biomolecules to form a physical and functional 3D living structures [4]. Bio-inks, prepared from natural or synthetic polymers, are expected to maintain structural integrity during the printing process.

A biodegradable material is defined as a material that is able to decompose under the influence of micro-organisms and their enzymes as well as under the influence of specific chemical substances. Biocompatibility and biodegradability of materials are two most important prerequisites for their use as a scaffold. These materials have to support cell attachment, migration and other basic cellular functions [5].

Polylactic acid (PLA) is a polymer with biodegradable properties. It ranks among the group of polymers which are obtained by production from renewable sources. The chemical structure of PLA is shown in Fig. 1.

PLA is a hard, inflexible and colourless polymer. The main advantage in biomedical use is its zero toxicity in the process of degradation in the biological organism. PLA is one of the few polymers that degrades into a basic monomer, from which is possible to recreate PLA polymer.

In the medical field PLA is mainly employed for medical implants in the form of anchors, screws, plates, pins, rods and as a mesh. Depending on the exact type used, it breaks down inside the body within 6 months to 2 years.

The melting point of PLA can be modified in the range of 130–220 °C. PLA density is 1.24 g·mm^{-3}, which is higher than the density of common polymers (0.8–1.1 g·mm^{-3}).

Polyhydroxybutyrate (PHB) is classified as polyhydroxyalkanoate (PHAs), which is a natural macromolecular substance. PHB represents the simplest polyalkanoate. The chemical structure is shown in Fig. 2.

The sources of a PHB are cells. To obtain PHB from the cells it is necessary to break down the cell membrane. Biodegradation of PHB takes place in two phases. In the first an enzyme connects to the polymer, and the second occurs via hydrolytic cleavage. The process of hydrolytic cleavage is affected by several factors, e.g. molar weight and crystallinity.

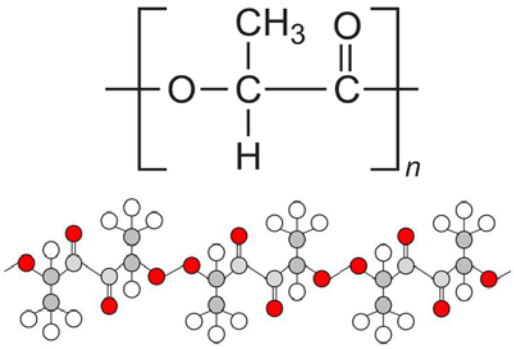

Fig. 1: Chemical structure of PLA.

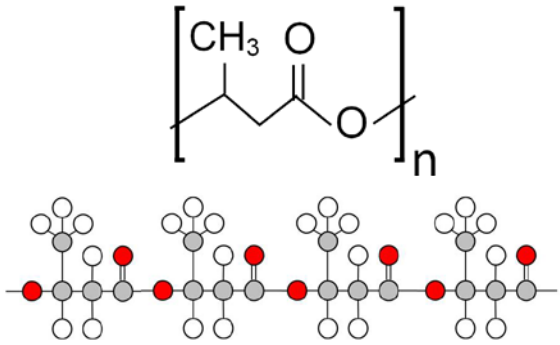

Fig. 2: Chemical structure of PHB.

In general, the scaffold should be fabricated from a highly biocompatible material which does not have the potential to trigger an immunological or clinically detectable body reaction [6]. Furthermore, a polymer scaffold material has to be chosen that will degrade and resorb at a controlled rate at the same time as the specific tissue cells seeded into the 3-D construct attach and proliferate.

Determination of the properties of polymers designated for bioprinting is necessary in order to understand how their characteristics affect the process of printing and the biocompatibility of the material. Design and production of bio-inks is affected by their physical and chemical properties, as these have an impact on the function, integrity and shape of the material, their viscoelasticity and their behaviour in different environment.

The main focus of this study was set on few important parameters of the printing process.

First step was to determine PLA/PHB material behaviour during the printing process. It is well known, this material is used as food packaging material for its biocompatibility and biodegradation properties, therefore manufacturing of suitable implant has big potential for many applications. At the beginning of the experiment, it was necessary to evaluate material behaviour during extrusion based bioprinting

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technology, while maintaining constant conditions of the printing process such as platform temperature, printing pressure, speed of printing and drying conditions. Cubic samples were designed and printed under those conditions and geometric properties were determined by metrotomography. These properties refer to material behaviour during the printing process.

Second step was to evaluate the influence of temperature and time on material viscosity. During processing and printing of material the degradation of material has been spotted due to high thermal sensitivity of PHB components of the mixture. This parameter was monitored during specified conditions and comparison of thermal degradation of dried and non-dried material was done.

Third step was to evaluate the viscoelasticity of material. Evaluation of viscoelasticity of material helps to set optimal parameters for 3D printing of PLA/PHB material.

Last step was to determine best parameters for the printing process of PLA/PHB material using 3D Bioplotter (Envisiontec, Germany) which could be helpful for next applications in bioprinting field.

Materials and methods

Design of test samples

For this study the test samples were manufactured in the shape of a cube with dimensions 10×10×10 mm. Design of test samples was performed in CAD software Solidworks 2013 (Dassault Systemes, France) and is shown in Fig. 3. Preparation of test samples for 3D printing process was performed in Bioplotter RP 3.0 from Envisiontec company (Fig. 5).

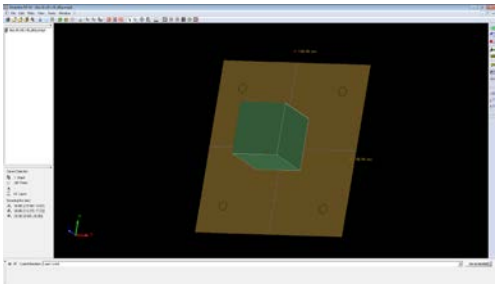

Fig. 3: Software design of printed samples.

Materials

Since cells and bioactive molecules respond to external environmental cues, it is important to determine whether processing of the material has an effect on the biological components in the scaffold, specifically on their morphology, composition and function [7]. Polymerization reactions, temperature, pressure, shear stress and deposition force are some of most important external processing conditions that could have an impact on cell viability and behavior [8].

The PLA + PHB material used was supplied by the Slovak Technical University in Bratislava. This material was in granulated form. Before the printing process, it is recommended to dry material. The process of drying was done in a drying oven at a stable temperature of 80 °C for 90 minutes. To determine the behaviour and difference between dried and non-dried material, a second group of the test samples were printed from material that had not been dried.

The material was inserted into a high temperature cartridge, with needle diameter of 0.6 mm used for each sample throughout the entire study.

Methods

Printing process

Extrusion-based bioprinting (EBB) (Fig. 4) was used to prepare test scaffolds for the experiment. EBB deposit materials using mechanical pressure or air, which is applied to the syringes or cartridges. The material is deposited in a line while the syringe or cartridge moves horizontally. It refers to a set of extrusion-based layered manufacturing processes capable of creating digitally controlled 3D patterns and constructs [9]. The extruded strands run parallel to each other, and the porosity of the final scaffold is defined by the distance between the strands. This process is finished when all layers of the CAD model are produced.

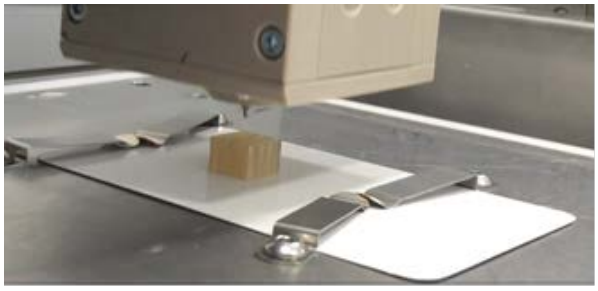

Fig. 4: Extrusion-based 3D printing.

Fig. 5: Bioplotter used for printing process.

Geometry of cube samples

Properties of PLA + PHB material had been considered to be highly dependent on processing of material such as drying before the printing process and

heating time in the cartridge needed for material to melt.

The inner structure of the cubic samples was set so that the layers were mutually turned 90 degrees. Cubic samples, shown in Fig. 6, were printed under pressures between 4–5 bars and at a speed between 2–9 mm·s^{-1} without any pores.

Fig. 6: 3D scaffold sample.

This unstable material behaviour was tested by printing samples of testing strands from non-dried and dried material at 3 different speeds (2/3/4 mm·s^{-1}) every 20 minutes. This testing showed the material fluidity due to the effect of temperature and time between the dried and non-dried samples.

Determining the geometry of the samples

Metrotomography was used for the purposes of analysis of geometry. Scaffold scanning was focused on the dimensions of samples and their porosity. Cross section images were created in the middle of cube dimension (5 mm).

Calculation of viscosity

Viscosity is a property that depends on the temperature and rate of degradation. Viscosity is determined experimentally. In addition, there are several types of materials, and this leads to several definitions of viscosity.

The main types of viscosity are:
Dynamic,
Kinematic,
Relative,
Apparent.

The mentioned formulas were applied to calculate the apparent viscosity of the polymer blends where the viscosity is affected by the shear rate [10]. The formulas are typically described for 3D printing of melted material passing through a circular cross-section of the nozzle, and therefore the formulas are derived for capillometry. This is how the material behaves in 3D printing.

Apparent viscosity η_z (1) relates to materials which do not have a constant viscosity. The constant parameter is temperature. Apparent viscosity η_z is determined as the ratio of shear stress τ [Pa] vs. the shear rate γ [s^{-1}] [10, 11, 12].

$$\eta_Z = \frac{\tau}{\gamma} \text{ [Pa·s}^{-1}\text{]} \quad (1)$$

Shear stress τ (2) is defined as the quotient of applied pressure on a needle p [Pa] and its radius r [mm] required to double the length of the needle l [mm] [10, 11, 12].

$$\tau = \frac{p \cdot r}{2 \cdot l} \text{ [Pa]} \quad (2)$$

Shear rate γ (3) is defined as the quotient of volume flow quadruplicating Q [mm^3] per 1 second to the constant π and the third power of the needle radius r [mm] [10, 11, 12].

$$\gamma = \frac{4 \cdot Q}{\pi \cdot r^3} \text{ [s}^{-1}\text{]} \quad (3)$$

To determinate the viscosity of the material it was necessary to specify the volume flow of PLA + PHB. This value was specified by CT scanning of the printed strands. Using metrotomography the width and height of the strand for calculation of the value per 1 second were determined (Fig. 7, Fig. 8).

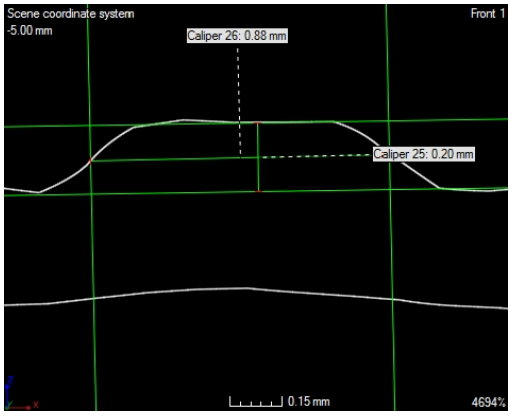

Fig. 7: Cross-section of strand.

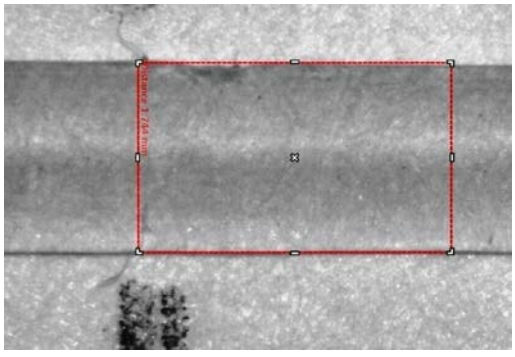

Fig. 8: Cross-section of strand.

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Results

After scanning all of the printed scaffolds it was apparent that there are many factors that influence the final quality of the printed part. The Fig. 9 shows four images: the top left shows the base of the scaffold, the top right picture shows the frontal cut, the bottom left image shows the sagittal cut and the fourth image shows a 3D image of the scaffold.

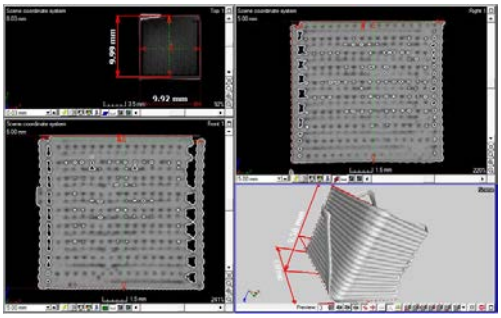

Fig. 9: Metrotomography scan.

The results showed more stable behaviour of the dried material. The dimensions of the first layer (base) were in all the samples above the setpoint (10 mm). This fact was influenced by the building-platform temperature (50 °C). If heating of the platform would not be done, the material would not attach to the surface of the platform. The quality of the walls of the cube and defects was influenced by the behaviour of the material. It has been confirmed that each sample printed from non-dried material had unequal walls that were not straight, Fig. 10. This was caused by the unstable behaviour of non-dried material.

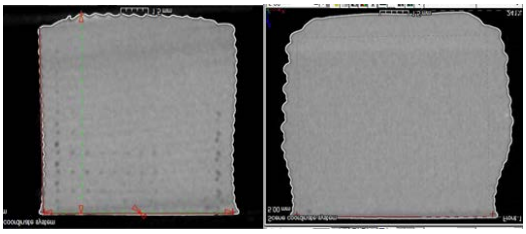

Fig. 10: Unequal walls of samples.

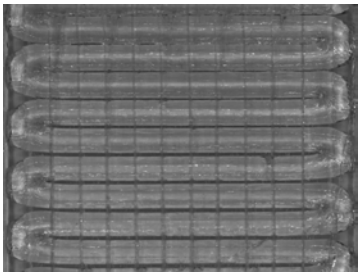

Fig. 11: Inner structure of samples.

The inner structure was influenced by the printing speed and the distance between the strands. This distance had to be changed because of the degradation process, which caused changes in the width of the strand. The strand width increases over time. The inner structure is shown in Fig. 11.

Time determination of heating of non-dried material

A testing strand was printed every 20 minutes as is shown in Fig. 12. During this process a change in the colour of each adjacent strand was evident as well as an increase in its width. Material degradation was confirmed in all non-dried samples after 80–100 minutes. The influence of the printing speed was negligible. The height and width of the samples were measured in three different places on the strand.

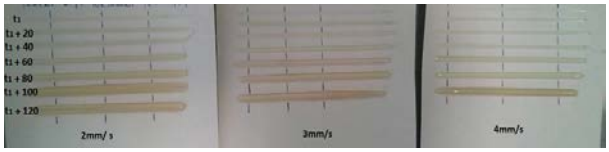

Fig. 12: Strands printed every 20 minutes.

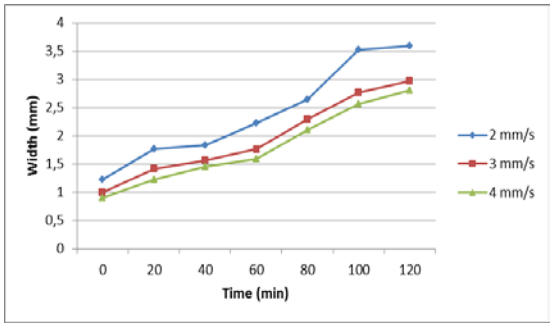

Fig. 13: Dependence of the velocity on the width of extruded fibres for non-dried material.

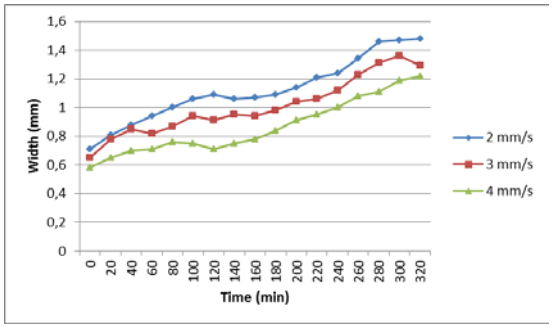

Fig. 14: The velocity dependence on width of extruded fibres for dried material.

The influence of the printing speed was negligible, both for non-dried and dried material. Material degradation was confirmed in all dried samples after more than 200 minutes. The height and width of the samples were measured in three different places on the strand. The comparison of Fig. 13 and Fig. 14 shows more stable behaviour of dried material, making it more suitable for printing process using Bioplotter.

The Fig. 15 represents a comparison of the change in the average fibres width of dried and non-dried material resulting in a visible higher stability of the dried material.

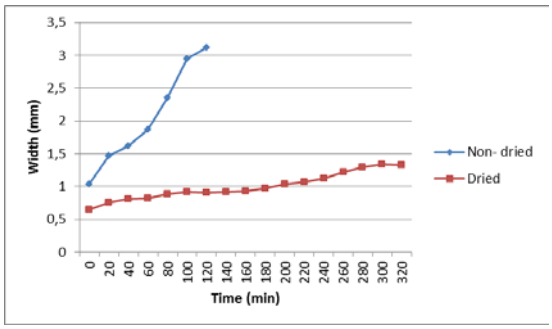

Fig. 15: A change in the fiber width in time of dried and non-dried material.

Determination of viscoelasticity

Over time the volume flow is increased, and this parameter was necessary for calculating the viscosity of PLA + PHB. As the graph shows, the printed strands changed their volume at every speed on the timeframe of 0–200 minutes (Fig.16, Fig. 17). The change of the real volume interprets the interval from 0.3 mm^3 (volume at time t1 at a speed of 2 $mm \cdot s^{-1}$) to 0.75 mm^3 (volume at time t1 + 200 min at a speed of 4 $mm \cdot s^{-1}$). After this timeframe, the material properties of the printed material began to visibly change. This finding shows the change in properties (width, colour) after the time t1 + 200 minutes for the dried material and t1 + 100 minutes for the non-dried material.

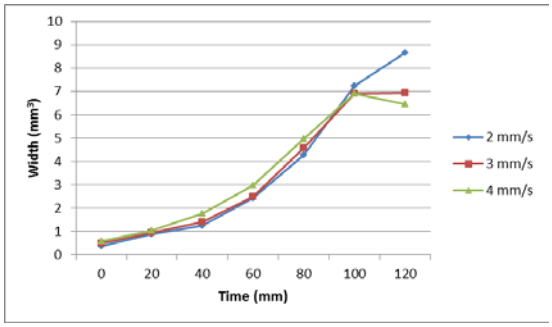

Fig. 16: Volume flow depending on time for non-dried material.

The Fig. 18 represents a comparison of the change in the average fibres volume flow of dried and non-dried material. The result is an evident higher stability of the dried material.

The viscosity of the material changes, and its value is different for each time period of testing. Viscosity is calculated for each time period from t1 = 0 minutes, t2 = t1 + 20 min, t3 = t2 + 20 min, etc. The constant values are π, the needle radius and the needle length. The plot shows the dependence of viscosity on time.

The viscosity of PLA + PHB showed a significant decreasing trend, which means that the material becomes more liquid with time. Fig. 19 shows the viscosity dependence on time for dried material.

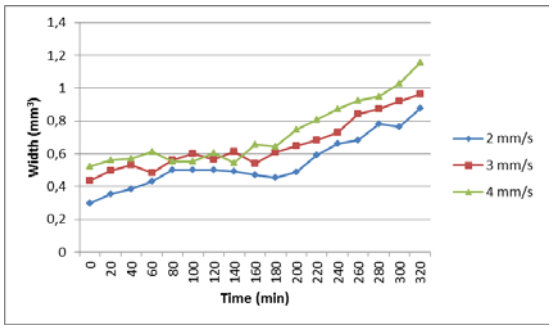

Fig. 17: Volume flow depending on time for dried material.

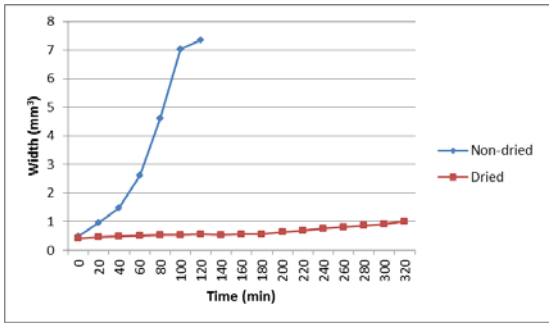

Fig. 18: Comparison of the volume flow of dried and non-dried material.

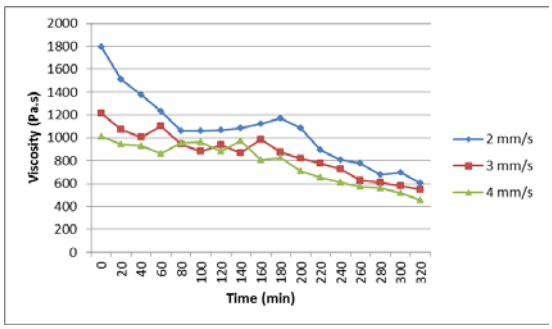

Fig. 19: Dependence of viscosity on time for dried material.

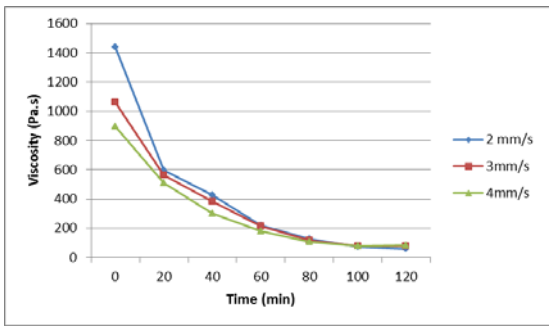

Fig. 20: Dependence of viscosity dependence on time for non-dried material.

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Non-dried material loses its properties needed for printing. It is evident that non-dried material viscosity value is over 80 minutes close to zero, which means the material is in the form of fluid (Fig. 20).

The change of viscosity of dried and non-dried material is shown in Fig. 21. The viscosity of non-dried material drops more rapidly than viscosity of dried material what leads to short using time during printing process.

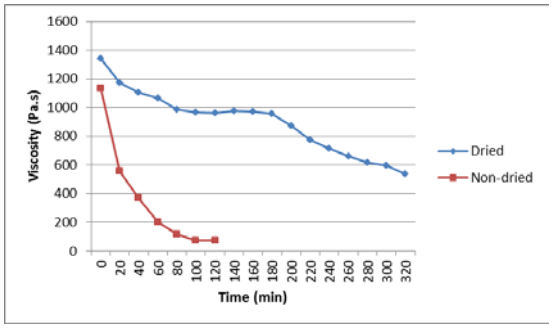

Fig. 21: Comparison of viscosity change during printing time for dried and non-dried material.

Discussion

Biodegradation is part of natural waste management and recycling system. It is a natural process necessary to keep our planet clean and healthy. The biodegradable materials such as polylactic acid and polyhydroxybutyrate (PLA + PHB) seem to be suitable candidates for various applications in bioadditive manufacturing. Ideally, 3D scaffolds should be highly porous, have well interconnected pore networks and have consistent and adequate pore size for cell migration and infiltration [13]. Scaffold architecture design can significantly influence both mechanical property and cell behaviour [14].

The results of this study are useful mainly for 3D printing devices which are using extrusion based technology, including Bioplotter (EnvisionTEC, Germany). It is caused by the fact, that the whole volume of material in the cartridge is being heated for all the time during the printing process. In different types of 3D printing technologies (for example Fused Filament Fabrication technologies) only a small amount of material passing through the needle is heated, which is immediately printed on building platform. The material is not exposed to high temperature for a long time, so thermal degradation is absent. It is necessary to define optimal conditions and parameters of pre-processing and printing by Bioplotter, in order to print material without any changes in its structure. Thermal degradation of PLA/PHB is generally known material property. Abdelwahab and co. [15] demonstrate thermal, mechanical and morphological properties of PLA/PHB mixture, where samples of material were heated on 510 °C (10 °C·min^{-1}) and using thermogravimetric analysis, it was conducted, that thermal properties of the mixtures could be significantly affected by the crystallization characteristics of PLA and PHB. Peelman and co. [16] also investigated heat resistance of new biobased polymeric material. The creation of copolymers can also ameliorate the heat resistance of a polymer. The copolymer PHBV (11 mol % HV into PHB) decreases Tm from 175 to 157 °C and Tg from 9 to 2 °C. The lower Tm means that the processing can be performed at temperatures further away from the degradation temperature than for pure PHB, but Tm is still high enough to not compromise its use as a packaging material (temperatures up to 120 °C).

Conclusion

It is possible to conclude a positive effect of drying the material in case of using it for extrusion based bioprinting. The degradation process during the printing of the material is more observable in the non-dried material than in the dried material. Ideal printing time for dried material is approximately up to 200 minutes after insertion of material into the cartridge. As compared with the non-dried material, which degraded after 60–80 minutes, the dried material is more suitable for printing process. The positive effect of material drying was confirmed by printed samples and shapes, sizes and dimensions. Determination of the viscosity was confirmed by the width of the fibre both from the dried and non-dried PLA-PHB material. According to time effect on the viscosity, the non-dried material shows a significant decrease as the material becomes more liquid. In the case of the dried material constant viscosity was obtained for a longer time (200 minutes). After this point, the viscosity decreased and the material became more liquid, thus lost the properties suitable for printing. According to this study the most suitable conditions for printing process using Bioplotter are to use dried material with printing time approximately up to 200 minutes. The pressure and speed depends on needle diameter. In this study the needle diameter of 0.6 mm was used, which enables printing process with 5 bar pressure and 2–3 mm·s^{-1} speed.

Recent developments in hard tissue reconstruction have shown important advances in both the materials and methods used. While autogenous tissue is still considered to be the gold standard for these reconstructions, the harvesting procedure remains tedious and in many cases causes significant donor site damaging or even death. These limitations have subsequently led to the development of less invasive techniques such as 3-D bioprinting that could offer possibilities to manufacture patient-tailored bioactive tissue constructs for hard tissue replacement. At

present there are many ways to create this kind of replacements as there is a wide range of 3-D printing technologies on the market. One of the major manufacturers of 3-D printers and also manufacturer of 3-D Bioplotter is Envisiontec company. This particular machine represents the idea of the possibility of 3-D bioprinting with no limitations in the choice of materials and parameters of the printing process. Advances in research of materials and methods in tissue engineering will certainly bring new approaches and ways to create fully functional tissues of the human body using 3-D bioprinting.

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References

[1] Bose, S., Roy, M., Bandyopadhyay, A.: *Trends Biotechnol*. 30 (2012) 546–554.

[2] Pan, T., Song, W.: *3D Bioplotting of Gelatin/Alginate Scaffolds for Tissue Engineering: Influence of Crosslinking Degree and Pore Architecture on Physicochemical Properties.* Journal of Materials Science & Technology. 2016(32), 889–900.

[3] Melchels, F. P. W., Domingos, M. A. N., Klein, T. J. , Malda, J., Bartolo, P. J. and Hutmacher, D. W.: *Additive Manufacturing of Tissues and Organs.* Progress in Polymer Science, 2012, 37, 1079–1104.

[4] Zhang, L. G.: *3D Bioprinting and Nanotechnology in Tissue Engineering and Regenerative Medicine*, 1st Edition. 1. Elsevier, 2015. ISBN 9780128005477.

[5] Chan, B. P., Leong, K. W.: *Scaffolding in tissue engineering: general approaches and tissue-specific considerations.* European Spine Journal 17 (4), 2008, 467–479.

[6] Stark, G. B., Horch, R., Tancos, E. (Eds.): *Biological matrices and tissue reconstruction*, Springer, Heidelberg, Germany (1998), pp. 197–206.

[7] Campbell, P. G., Weiss, L. E.: *Tissue engineering with the aid of inkjet printers.* Expert Opin Biol Ther. 2007;7(8).

[8] Trachtenberg, J. E.: *Principles of Tissue Engineering.* Fourth Edition: Polymer Scaffold Fabrication. 4. Elsevier Inc., 2013. ISBN 9780123983589.

[9] Mandrycky, C.: *3D bioprinting for engineering complex tissues.* Biotechnology Advances. 2016, (34), 422–434.

[10] Alexy, P.: *Procesy spracovania polymérov*. Slovenská technická univerzita v Bratislave, STU, Slovensko, 2011. ISBN: 9788022734707.

[11] Chang, D. H: *Rheology and Processing of Polymeric Materials.* Volume 1, Polymer Rheology, 2007. ISBN 978-0-19-518782-3.

[12] Thomas, G. M.: *The Rheology Handbook*. 4th Edition, 2014. ISBN 978-3-86630-842-8.

[13] An, J.: *Design and 3D Printing of Scaffolds and Tissues. Engineering.* 2015, 1(2), 261–268.

[14] Hollister, S. J.: *Porous scaffold design for tissue engineering.* Nat. Mater., 2005, 4(7): 518–524.

[15] Abdelwahaba, M. A., Flynnb, A., Chiouc, B.-S., Imamc, S., Ortsc, W., Chiellini, E.: *Thermal, mechanical and morphological characterization of plasticized PLA–PHB mixtures.* Polymer Degradation and Stability 97, (2012) 1822e1828.

[16] Peelman, N., Ragaert, P., Ragaert, K., De Meulenaer, B., Devlieghere, F. and Cardon, L.: *Heat resistance of new biobased polymeric materials, focusing on starch, cellulose, PLA, and PHA.* J. Appl. Polym. Sci., 2015, 132, 42305. doi: 10.1002/app.42305.

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