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Procedia CIRP 89 (2020) 104–109

www.elsevier.com/locate/procedia

CIRP BioManufacturing Conference 2019

Bioprinting process optimization: evaluation of parameters influence on the extrusion of inorganic polymers

P.S. Ginestra[a*], R. Rovetta[a], A. Fiorentino[a], E. Ceretti[a]

[a]Department of Mechanical and Industrial Engineering- University of Brescia – Via Branze 38, 25123 Brescia - Italy

* Corresponding author. Tel.: +390303715538; *E-mail address:* paola.ginestra@unibs.it

Abstract

Bioprinting consists in an innovative approach able to improve the current techniques of bioregeneration in the medical field through the extrusion of cell-loaded bioinks. Its main advantage is the customization to reduce post-operative complications on the patient, as it can be produced from his own cells. The success of bioprinting is determined by the printing parameters but, above all, by the materials. The goal of this work was to define a range of parameters, in order to achieve the highest printing stability, in terms of the quality of the Bioplotter® Silicone TG in relation to process conditions used.

Keywords: 3D printing, bioprinting, scaffold, polymers.

1. Introduction

3D printers are able to produce physical objects, starting from a three-dimensional model, through the deposition of several layers of material [1-3]. The materials used in 3D printing are mainly plastic, metallic and polymeric, but living cells can also be used to artificially reproduce tissues, blood vessels and organs [4]. Among the several biomanufacturing techniques [5-7], 3D printing technologies faced a rapid expansion of applications in medicine and tissue engineering for the fabrication of scaffolds [8-10]; while bioprinting, through the use of biocompatible materials, has been developed for the production of different natural tissues, including skin, cartilage, bone, neural and muscle tissues [11-13]. Bioprinting consists in an innovative approach able to improve the current techniques of bioregeneration in the medical field through the extrusion of cell-loaded bioinks. This innovative approach is improving the current techniques of bioregeneration in the medical field due to the high degree of customization involved, which allows to reduce post-operative complications on the patient, as it can be treated with his own cells or specific stem cells [14,15]. Extrusion-based bioprinting (EBB) can print a wide range of biomaterials, including solutions, pastes or dispersions but is applicable only to viscous liquids and has limitation on complexity of shapes. The success of the EBB is determined by the technique and the printing parameters but, above all, by the materials. Thus, the optimization of the extrusion process is required to identify the potential applications and performances of a new biocompatible material. One of the most important feature of 3D Bioprinting is the bioink, which is the bio-printable material used in the process. This material should be biocompatible, in order to accommodate live cells, and mechanically stable after printing. The biomaterials used, either encapsulating cells or loaded with cells later on, are generally injected into metal or plastic syringes and extruded with the application of a proper pressure. The Envisiontec 3D Bioplotter® System is a versatile machine tool, mainly used to prototype

10.1016/j.procir.2020.05.125

a great variety of biomaterials from 3D CAD models. The Bioplotter® is a multi-part and multi-material system using an automatic tool changer and multiple print heads, with individual temperature control. A liquid, melt, paste or gel is dispensed from a material cartridge through a needle tip from a 3-axis system to create a 3D object. It is designed for use in a sterile environment within a biosafety cabinet; indeed, the equipment includes built-in particle and sterile filters for the input compressed air [16].

The Envisiontec Bioplotter® is able to print a great variety of biomaterials, but the first material tested with the first 3D-Bioplotter prototype in 2000 was Silicone [17,18]. Silicone has been on the market for many years and is currently used in many processes, both industrial and domestic. In fact, silicone is a polymer made from silica contained in sand and rocks, and it can be found in solid and liquid form, depending on the length of the chain and their cross-linking degree. Silicon is very versatile, economical and different textures can be tested according to the requirements of the final application, especially in medical fields. This paper is focused on the evaluation of the material characterization methodology developed for the Bioplotter® equipment. More specifically, the goal of this work was to define a range of process parameters, in order to achieve the highest printing stability, in terms of the quality of the 3D-Bioplotter® Silicone in relation to the nozzle dimensions and process conditions used. In particular, the influence of extrusion pressure and speed on precision and uniformity of the produced components was analysed, so to optimize the process and to identify the influence of these parameters on filament formation and deposition too. The realized strands were observed under optical microscope and measured in terms of diameter of the deposited wire. The statistical analysis of the results was carried out to consider the effects of each factor and their interactions on the final geometry to gain a better reproducibility and repeatability of the part. The proposed procedure can be considered a valid approach for the optimization of the process parameters for the extrusion of new developed materials.

2. Definition of the tuning parameters

In this section, the experimental method for the extrusion process optimization is reported. In particular, different tests were carried out in order to define a promising area for the obtainment of a diameter of the silicon extruded filament in relation to the diameter of the used nozzle. The extruded filaments were produced by the 3D-Bioplotter® printer whose range of process parameters is reported in Table 1. The Bioplotter® printer works as a material extruder where A liquid, melt, paste or gel is dispensed from a material cartridge through a needle tip from a 3-axis system to create a 3D object.

The key feature of the 3D-Bioplotter is the flexibility in the choice of materials. In particular, the Bioplotter® allows the evaluation of the extrusion properties of new materials and the setting of the processing parameters by using the Material Parameter Tuning procedure. The needle offset defines the starting spacing between the needle tip and the platform at the beginning of the extrusion process. Values between 50% and 100% of the inner diameter of the needle tip can be chosen, dependent on the materials and platform substrate.

Table 1 Bioplotter® manufacturer series parameters.

Process parameter	Range values
Platform temperature (T)	-10°C - 80°C
Heads #	Low: 0°C -70°C High: 30°C - 250°C
Cartridge sizes	10-30 ml
Build volume	150x150x140 mm^3
Speed (V)	0.1-150 mm/s
Pressure (P)	0.1-9.0 bar

The most important parameters that are considered in this work are the extrusion pressure and speed. Under Pressure Tuning Parameter a fixed speed can be selected, as well as the minimum and maximum pressures. Five strands are printed on the back half of a 100x100 mm surface. Similarly, the Speed Tuning Parameter can be used at a fixed Pressure with Min and Max Speed values. These five strands are printed in the front half of the 100x100 mm surface.

The Technical Grade Silicone (TG, Envisiontech®, Silicone, viscosity=1,900,000mPs) was extruded with the printer low temperature head to evaluate the process stability.

The parameters of speed, pressure and offset in the Z direction were modified to achieve the optimal printing conditions considering the deposition and uniformity of the extruded filament. The following tests were performed at room temperature:

- Pressure tuning: speed kept fixed at the intermediate value of the tuning speed range, while the pressure range is divided into 5 values of the imposed range values.
- Speed tuning: pressure kept fixed at the intermediate value of the tuning pressure range, while the speed range is divided into 5 values of the imposed range values.

The tuning configurations imposed were chosen in order to find the optimal value of the offset in the Z direction for this type of material, varying pressure and speed of extrusion (Table 2, 3 and 4). Each tuning is performed by fixing one of the values of the Zoffset (between 0.35 and 0.38 mm) and varying the pressure values at a certain speed (10, 13 and 15 mm/s) or varying the speed values at a certain pressure (2, 2.3 and 2.5 bar)-. At each value of the Zoffest, two tests were carried out: one for the pressure tuning at a fixed speed (i-e. 10mm/s for tuning#1) and one for the speed tuning at a fixed pressure (i.e. 2.3 bar for tuning#1).The first tuning (tuning #1) configuration was imposed according to the material datasheet provided while the second tuning (tuning#2) was designed to narrow the range of parameters and find the right Zoffset. The third tuning (tuning #3) was then carried out for the optimization of the pressure and speed parameters of the process. Three repetitions were fabricated for each process parameters set.

Table 2 Designed tuning configuration (#1). The pressure values were varied between 0.5 and 4 bars at a fixed speed of 10 mm/s while the speed values were varied between 5 and 15 mm/s at a fixed pressure of 2.3 bar).

Tuning #1		
Pressure [bar]	Speed [mm/s]	Z_{offset} (mm)
from 0.5	from 5	0.35
to 4	to 15	0.36
V= 10 mm/s	P=2.3 bar	0.37
		0.38

Table 3 Designed tuning configuration (#2). The pressure values were varied between 2 and 3 bars at a fixed speed of 13 mm/s while the speed values were varied between 12 and 14 mm/s at a fixed pressure of 2.5 bar).

Tuning #2		
Pressure [bar]	Speed [mm/s]	Z_{offset} (mm)
from 2	from 12	0.35
to 3	to 14	0.36
V= 13 mm/s	P=2.5 bar	0.37
		0.38

Table 4 Designed tuning configuration (#3). The pressure values were varied between 1.8 and 2.2 bars at a fixed speed of 15 mm/s while the speed values were varied between 14 and 16 mm/s at a fixed pressure of 2 bar).

Tuning #3		
Pressure [bar]	Speed [mm/s]	Z_{offset} (mm)
from 1.8	from 14	
to 2.2	to 16	0.36
V= 15 mm/s	P=2 bar	

3. Extrusion characterization

To understand if the parameters involved in the process reflected our expectations, for each test performed, the diameter of the printed strands was measured, using Mitutoyo microscope, and compared with the diameter of the nozzle used: 0.4 mm. The microscope images were processed with ImageJ software [19] to measure the diameters of the filaments. The measurements were taken on different areas of the strand to consider the uniformity of the extrusion.

3.1. Tuning #1

The results of the tunings are reported in Figure 1 and 2 showing the diameters of the extruded filaments that were classified in ranges of values closer to the diameter of the nozzle (d_0=0.4mm). The absolute value of the difference between the extruded filament (d) and the nozzle diameter (d_0) has been considered. In particular, the green color represents the values nearer to the nozzle diameter which can be considered optimal in terms of quality of the filament ($|d-d_0|\leq 0.05$), the yellow dots are considered acceptable values ($0.05< |d-d_0|\leq 0.1$), the orange dots represent a bad quality of the extruded filament ($|d-d_0|>0.1$) while the red ones are considered points of inapplicability of the printing (i.e. discontinuities, irregular printing, air bubbles).

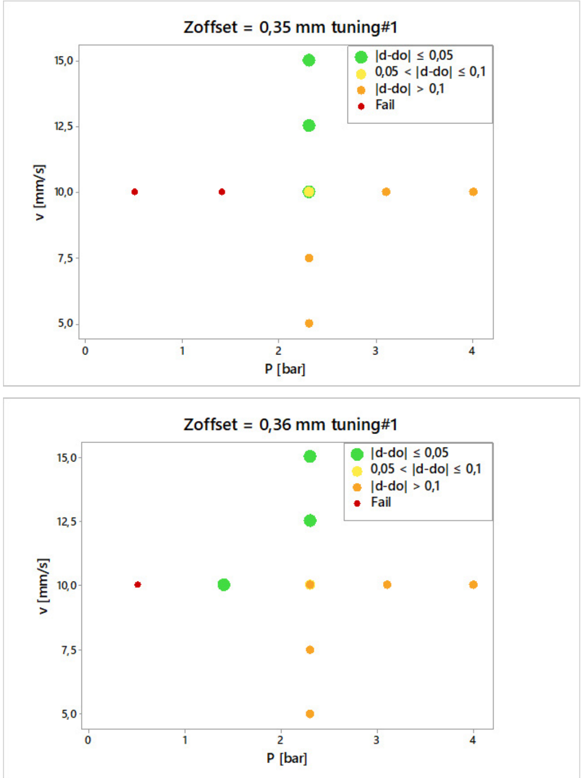

Fig. 1 Results of the tuning#1 considering offset values of 0.35 mm and 0.36 mm. The dots represent the value ranges of the diameter of the filament and each color correspond to a level of acceptation of the filament diameter values. In both cases, the optimal values were found at a pressure of 2.3 bar and a speed ≥ 10 mm/s.

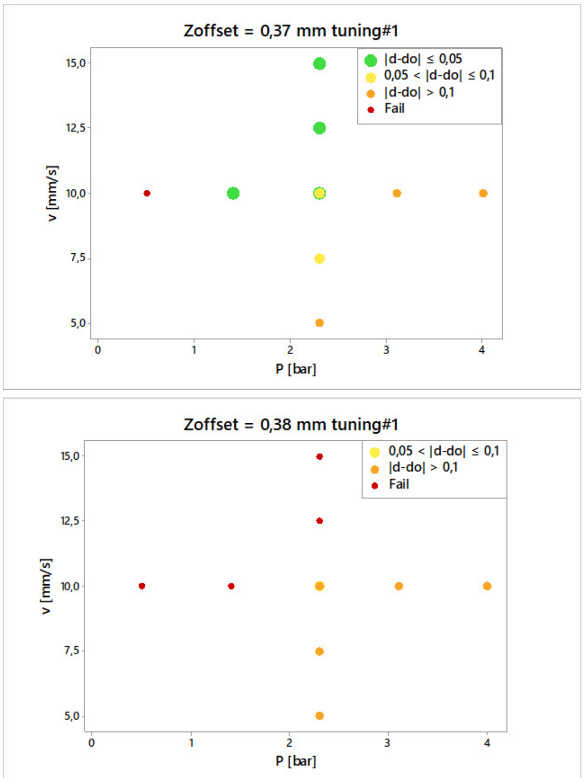

Fig. 2 Results of the tuning#1 considering offset values of 0.37 mm and 0.38 mm. The dots represent the value ranges of the diameter of the filament and each color correspond to a level of acceptation of the filament diameter values. While at a Zoffset of 0.37 mm the optimal values were find at a pressure of 2.3 bar and a speed ≥ 10 mm/s, at a Zoffset of 0.38 mm the diameter of the filament is printed in bad quality or tends to be not printable.

From the results of tuning#1 (Figure 1 and 2) it can be concluded that as the Z_{offset} increases, at low values of pressures and high speeds, the precision of the printed strands decreases, making the filament often not uniform, wavy or full of droplets. These tests are represented with red dots, which define the inapplicability of the printing process and consequently the measurement. At lower values of Zoffset (0.35 and 0.36 mm/s), the optimal values of diameter are found at higher values of speed (≥10 mm/s) due to the dragging force that prevent the compression of the filament. This effect is reduced at a Zoffset of 0.37 mm, where acceptable values can be found at lower speeds (≤ 10 mm/s). The same considerations are not valid for a Zoffset equal to 0.38 mm due to the inadequate values of the pressure that are not allowing enough deposition of material. Considering these results, higher values of speed and pressure were selected for the tuning#2 for obtaining filament diameters within the range of 0.4±0.1 mm with a speed higher than 12mm/s and a pressure higher than 2 bar.

3.2. Tuning #2

The tuning#2 was based on a different range of parameters due to the analysis of the results of tuning#1. In particular, the ranges of both pressure and speed parameters were restricted and the offset values considered in this case were amplified. From the graphs in Figure 3 and 4, as from the previous tests carried out, it can be noticed that the offset in the z direction, that allows to get to the most promising results, is 0.37 mm (92% nozzle diameter). Moreover, the diameters are closer to 0.4 with a variability of ±0.05 mm when the pressure is less than 2.25 bar and the speed higher than 12.5 mm/s.

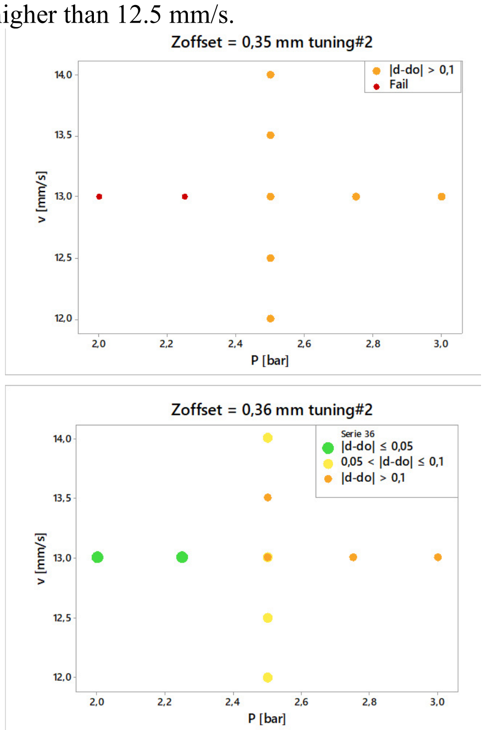

Fig. 3 Results of the tuning#2 considering offset values of 0.35 mm and 0.36 mm. The dots represent the value ranges of the diameter of the filament and each color correspond to a level of acceptation of the filament diameter values. In first case (Zoffset=0.35 mm), the quality of the filament results bad for every parameters combination, in the case of Zoffest=0.36 mm, the optimal values were found at a pressure ≤ 2.3 bar and a speed of 13 mm/s.

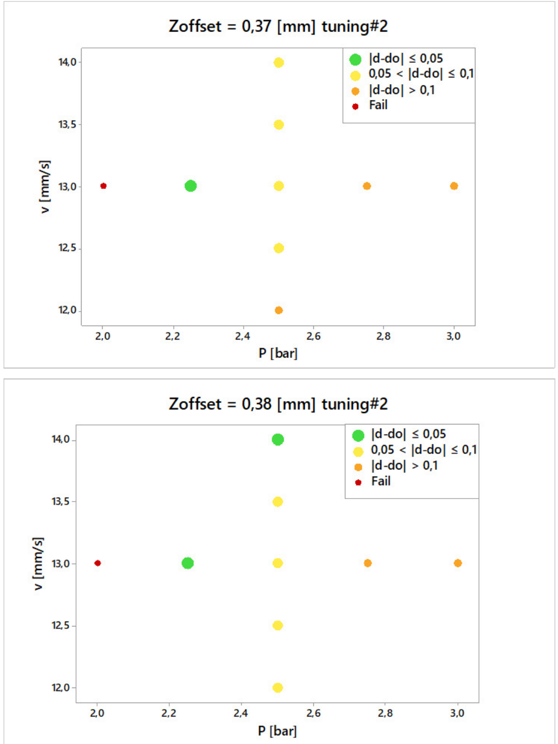

Fig. 4 Results of the tuning#2 considering offset values of 0.37 mm and 0.38 mm. The dots represent the value ranges of the diameter of the filament and each color correspond to a level of acceptation of the filament diameter values. With a Zoffset of 0.37 mm, one optimal value was found at a pressure of 2.3 bar and a speed of 13 mm/s while with a Zoffset of 0.38 mm, two optimal values were found at a pressure of 2.3 bar and speed of 13 mm/s and at a pressure of 2.5 bar and a speed of 14 mm/s.

From the results of tuning#2 (Figure 3 and 4) it can be concluded that at a Z_{offset} of 0.35 mm, at low values of pressures, the precision of the printed strands decreases, making the filament often not uniform, wavy or full of droplets. As the pressure is increased, still not acceptable values of the diameters are measured. At higher values of Zoffset (0.37 and 0.38 mm/s), the optimal values of diameter are found at higher values of speed (≥10 mm/s) and pressures between 2.3 and 2.5 bar. This can be explained by the fact that these pressure values assure an adequate deposition of material and the speed values can be increased. The Zoffset of 0.36 mm offers a wider range of optimal values at lower pressures due to the right position of the needle tip in relation to the plate that influences the amount of material deposited. These results confirm the influence of the Zoffset on the parameters tunings to perform a process optimization. In particular, a Zoffset of 0.36 mm was chosen for the final tuning allowing the application of lower pressures values for the obtainment of the optimal filament quality.

3.3. Tuning #3

Tuning#3 has been carried out considering a fixed value of the Z_{offset} equal to 0.36 mm taking into account the results of tuning#2. The speed values were increased considering the trending of the diameter values resulted from tuning#1 and tuning#2 that showed an improving of the diameter values at higher speed (Fig. 1,2 and 4). Figure 5 reports the results of the measurements on the extruded filaments.

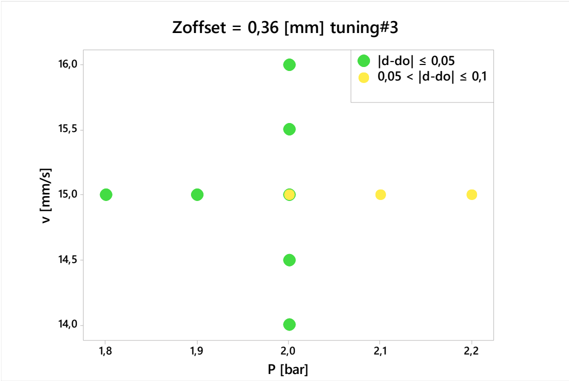

Fig. 5 Results of the tuning#3 considering an offset value of 0.36 mm. . The dots represent the value ranges of the diameter of the filament and each color correspond to a level of acceptation of the filament diameter values. In this case, the optimal values were found at a pressure ≤ 2 bar and a speed ≥ 14 mm/s.

From these last results, it can be concluded that the most suitable values to assure a diameter of the filament as closer as possible to the diameter of the selected nozzle are:

- 1.8 bar ≥ Pressure ≤ 2 bar
- Speed ≥ 15 mm/s

Nevertheless, at a speed of 15 mm/s, as the pressure increases, the precision of the printed strand decreases, and it moves increasingly away from the target value of 0.40 mm of the nozzle diameter. To show what was found by the tuning of the parameters, Figure 6 reports three examples of printed filaments during tuning#3 at fixed speed equal to 15mm/s and increasing pressure from 1.8 bars to 2.2 bar The filament diameter tends to increase with an increasing of the pressure value.

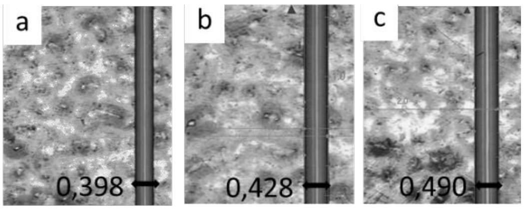

Fig. 6 Optical microscope images 1.5x of the silicon strands produced by the extrusion at a speed of 15mm/s and pressure of 1.8 (a), 2.0 (b) and 2.2 (c) bar.

It can be noticed that all the strands have no interruptions, waves or drops along the deposition area while, when the pressure is increased, the diameters of the strand starts to increase. The results are confirmed by the contour plot and the 3D scatter plot considering all the tunings performed with a Z_{offset} equal to 0.36 mm (Fig. 7).

These results confirm the advantages offered by the material tuning for the parameters optimization of the printing process of new materials.

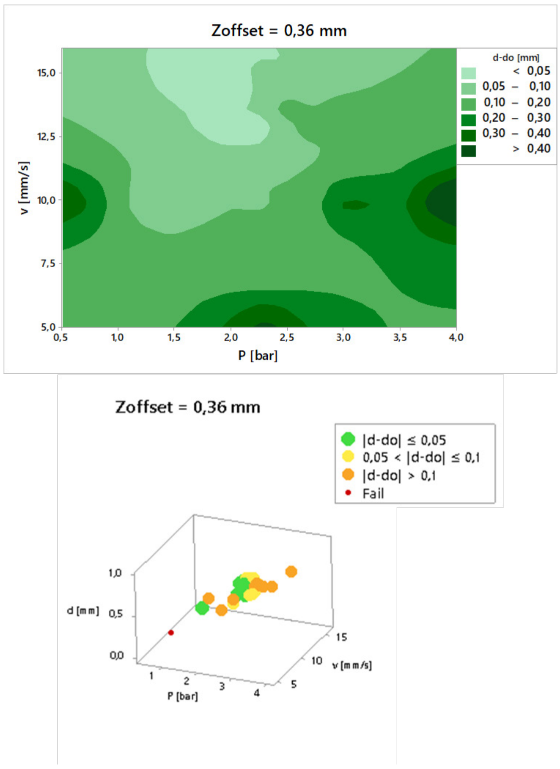

Fig. 7 Contour and scatterplot of d-d_0 as a function of different pressure and speed values. The contour plot helps in defining the optimal region of values corresponding to specific combinations of the parameters used for the process optimization (speed and pressure) while the scatterplot defines all the points considered in the analysis to identify the area that has been investigated.

4. Conclusions

The paper deals with the extrusion characterization of a new generation 3D printer: the 3D-Bioplotter® manufacturer series. The objective was focused on the evaluation of the tunings schemes that can be executed by the equipment for the characterization of a new material that has to be extruded. The process was analyzed by the extrusion of the TG Silicone provided by Envisiontec by using the low temperature head of the printer. In particular, the diameter of the extruded filament was analyzed under optical microscope to measure the variability of the diameter of the extruded silicone strands in relation to the diameter of the selected nozzle. Three tunings based on different combinations of pressure and speed used during the process were performed.

From the experimental results reported in this work it can be concluded that the offset in z direction influences the extruded diameter within a percentage of 10% while when the offset is kept constant, it is possible to define a process window corresponding to the extrusion stability for the specific analyzed material. In particular, the higher precision of the process is found when a pressure of 2 bars is chosen in relation to a speed between 14 and 16 mm/s. The dispense pressure is dependent on the material flow through the nozzle and must be adjusted to the rate at which the material hardens. A low dispense rate will allow the material to harden while still being affected by the needle tip, resulting in thinner and rounder strands. A dispense rate too fast relative to the hardening speed will result in wide, oval strands flattened by the material during the hardening period. A relatively low pressure (≤2 bars) is allowing the silicone to harden in the right time to lead to filaments with a diameter closer to the nozzle's diameter. The dispense rate must be adjusted to the XY movement speed. A wrong relation between dispense pressure and XY movement speed will cause the formation of defects on the deposited strands. In particular, with a dispense rate too high relative to the XY movement speed, too much material is dispensed and the strand starts to swell. The optimal situation was reached when the material leaves the nozzle and is immediately pressed against the plate (14 mm/s ≤V≤16 mm/s). The strand is only slightly deformed due to the type of material that is extruded (Fig. 7) but the strand is not stretched after being dispensed causing a delay in the deposition on the plate that would have led to droplet formation instead of round strands.

The procedure introduced in this work can be considered a valid methodology for the identification of the optimal process parameters for the testing of new materials. More specifically, this approach can be used for the testing of *ad hoc* designed materials not commercially available that need to be characterized to investigate and then tailor their extrusion process. The future investigations are going to be focused on the analysis of the data dispersion and the influence of the other process parameters such as the printing platform temperature. Moreover, the dimensions of the deposited strands during multi-layered printing tests are going to be studied in order to identify the process stability area and compare it to the one found for the deposition of one single strand.

Acknowledgements

The authors would like to acknowledge the support and the assistance of Prof. Luca Giorleo of University of Brescia.

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