INKJET PRINTING OF MATERIALS WITH RESISTANCE TO BACTERIAL ATTACHMENT

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Abstract

Biofilm formation on the surface of medical devices is a major source of health-care associated infections. The discovery of new materials that inherently avoid formation of such biofilms on their surface points the way to the fabrication of biofilm resistant devices, with the consequent reduction in the incidence rate of device centred infections and therefore a reduction in suffering and costs for health-care systems. Drop on Demand (DOD) Three Dimensional (3D) Inkjet Printing presents higher versatility than common techniques for printing biomaterials. One of the main representations of this enhanced versatility is polymerisation post-jetting, which provides a great range of printable polymers. The combination of such materials with inkjet printing could revolutionise the biomedical industry.

In this paper, the printability of four acrylates with resistance to bacterial attachment was assessed using the printability indicator or Z parameter. Three of the materials showed a value of Z within the printability range. The remainder displayed a Z value higher than the maximum suggested. However, this material was ejected with stability using a complex waveform designed for low viscosity inks. Drop spacing was optimised for each ink using PET and glass as substrates. The combination of printability optimisation together with ideal drop spacing allowed the construction of 3D structures of three of the four inks that were tested.

Introduction

Currently, one of the major problems found by clinicians is the high percentage of infections caused by bacterial films attached to them. These microbial colonies develop up to 1000 times higher tolerances to antibiotic treatment and the host immune system compared with their planktonic counterparts[1,2]. Most current strategies oriented to the reduction of biofilm-associated infections focus on the modification of existing materials used to manufacture medical devices. This approach is based on the incorporation of antibiotics[3] or other antimicrobials, such as silver salts, nitrofurazone, chlorhexidine, polymerized quaternary ammonium surfactants, antibacterial peptides and anionic nanoporous hydrogels[4-9]. These natural or synthetic chemicals kill the bacteria cell already attached to the device surface. Nevertheless, an alternative approach is to use of materials that inherently resist biofilm formation[10]. First trials included the use of poly(ethylene glycol) brushes[11] and zwitterionic polymers[12]. However, the discovery of new acrylates using high throughput materials discovery to identify polymers which resist bacterial attachment[13,14] have been shown to function in vitro and in vivo with potential to directly reduce device centred infections.

In the last decades, 3D printing has arisen as an alternative methodology for building highly complex customised devices. The direct flow from CAD design to manufacturing plant together with the high level of complexity reached in the parts make these techniques the ideal solution for industry, especially in the aeronautical and biomedical sectors.

In the case of biomedical applications, the most common polymers are often printed applying techniques[15] of extrusion or laser sintering. However, inkjet printing opens the door to the utilization of fluid inks as structural materials, this means the use of new polymers outside the range of the workable polymeric materials used in the two techniques mentioned previously. Other advantages of inkjet printing are its mechanism of the drop-on-demand (DOD) deposition, which increases the versatility of the process including accurate positioning of the politer-size drops, and the independence of the substrate.

The combination of these materials resistant to bacterial attachment with the versatility of inkjet printing could bring the biomedical field to another level. So, the aim of this work was the development and optimization of four new bacteria-free materials for inkjet printing using a DOD droplet generation.

The target monomers were selected among the range of acrylate- and methacrylate-derivatives showing best results as bacterial resistant materials, according to the publication[13,14] of Hook et al. The approach followed for this selection was: one acrylate monomer (A), one diacrylate monomer (B) structurally similar to A, one aliphatic acrylate (C) and a polar acrylate (D).

DOD droplet generation requires fluids with certain physical and mechanical characteristics[18]. These characteristics are reflected in different dimensionless groupings of physical constants, the most useful of which are the Reynolds (Re) (Equation 1), Weber (We) (Equation 2), and Ohnesorge (Oh) (Equation 3) numbers:

$$Re = \frac{v\,\rho\,r}{\mu} \tag{1}$$

$$We = \frac{v^2\,\rho\,r}{\gamma} \tag{2}$$

$$Oh = \frac{\sqrt{We}}{Re} \tag{3}$$

Where v is the velocity, r is the nozzle diameter, and ρ, μ and γ are the density, dynamic viscosity and surface tension of the fluid, respectively.

Fromm[19] defined the Z parameter (or printability indicator) as the appropriate constant to determine the printability of a certain fluid, where Z is defined as

$$Z = \frac{1}{Oh} = \frac{\sqrt{\rho\,r\,\gamma}}{\mu} \tag{4}$$

Reis and Derby[20] used numerical simulation of drop formation to propose the range for stable droplet formation.

$$1 < Z < 10$$

In the present paper, the printability parameter (Z) for the inks prepared from the target monomers will be assessed. 2,2-Dimethoxy-2-phenylacetophenone (DMPA) was used

as the photoinitiator for the post-deposition polymerisation reaction. Rheological data were collected from 25ºC to 60 or 70ºC, while the surface tension was measured at the corresponding printing temperature or in a range from 25 to 60ºC. Density values of pure monomers were used since it was assumed insignificant variation in the ink density when small amounts of initiator were added.

Experimental section

General Methods. Monomers and initiator were purchased from Aldrich Chemical Co. and used as received. The stirring process was carried out using an IKA RCT Basic IKAMAG Magnetic Stirrer (with Temperature Controller). A Malvern Kinexus Pro Rheometer equipped with a cone plate was used for viscosity measurement under shear rates from 10 s^{-1} and 1000 s^{-1}. Each measurement started at 25ºC with 5ºC increments up to 60 or 70ºC, depending on the monomer. A protocol of waiting 300 s after reaching the test temperature was set to ensure the ink was in a steady state condition. At each temperature point and shear rate, the viscosity was recorded at 5 s intervals within a 180 s test time. For the determination of surface tension a Kruss DSA100S was used, applying the pendant drop method. Printability tests were carried out in a Dimatix DMP-2800. The cartridges utilised in the experiments were characterised for a 21 μm nozzle diameter.

Ink Preparation. All inks were prepared by mixing 1% of DMPA, as photoinitiator, with the corresponding monomer, using amber vials. To help the solution, the mixture was placed on the stirrer for 10 min at 50ºC. A flow of N_2 was applied to the mixture for 10 min for degassing. Inks were kept in the dark for two days to release bubbles.

Sample Printing. A Dimatix DMP2800 was installed inside a glovebox with an O_2 sensor. A N_2 flow was circulated until the O_2 level was less than 1%. An UV light (365 nm, 3.5 J/cm^2) was assembled on to the Dimatix printhead carriage to carry out sample curing while printing.

Results and Discussion

As showed in the Z parameter equation (4), the most influential property in the printability of a certain ink is the viscosity. The values of viscosity for inks A, B and D are shown in Figure 1. For acrylate C, data found in the literature[21] was used: 1.5 cps (25ºC), which was too low for printing at ambient temperature, therefore further rheology assessment was not required. It was considered that the addition of only 1% of photoinitiator was not going to imply a significant increment of the value of this property. The selected printing temperatures and their corresponding viscosity values are shown in Table 1, where 100 s^{-1} of shear rate was established as a reference.

Ink	Temperature (°C)	Shear Viscosity (cP)	Surface Tension (mN/m)	Density (g/ml)	Z Parameter
A	40	9.4	35	1.09	3
B	70	12.9 m	31	1.1	2.1
C	25	1.5[21]	26[22]	0.89	14.7
D	60	13.7	38	1.16	2

Table 1: Values of printing temperature, shear viscosity, surface tension and density for each monomer

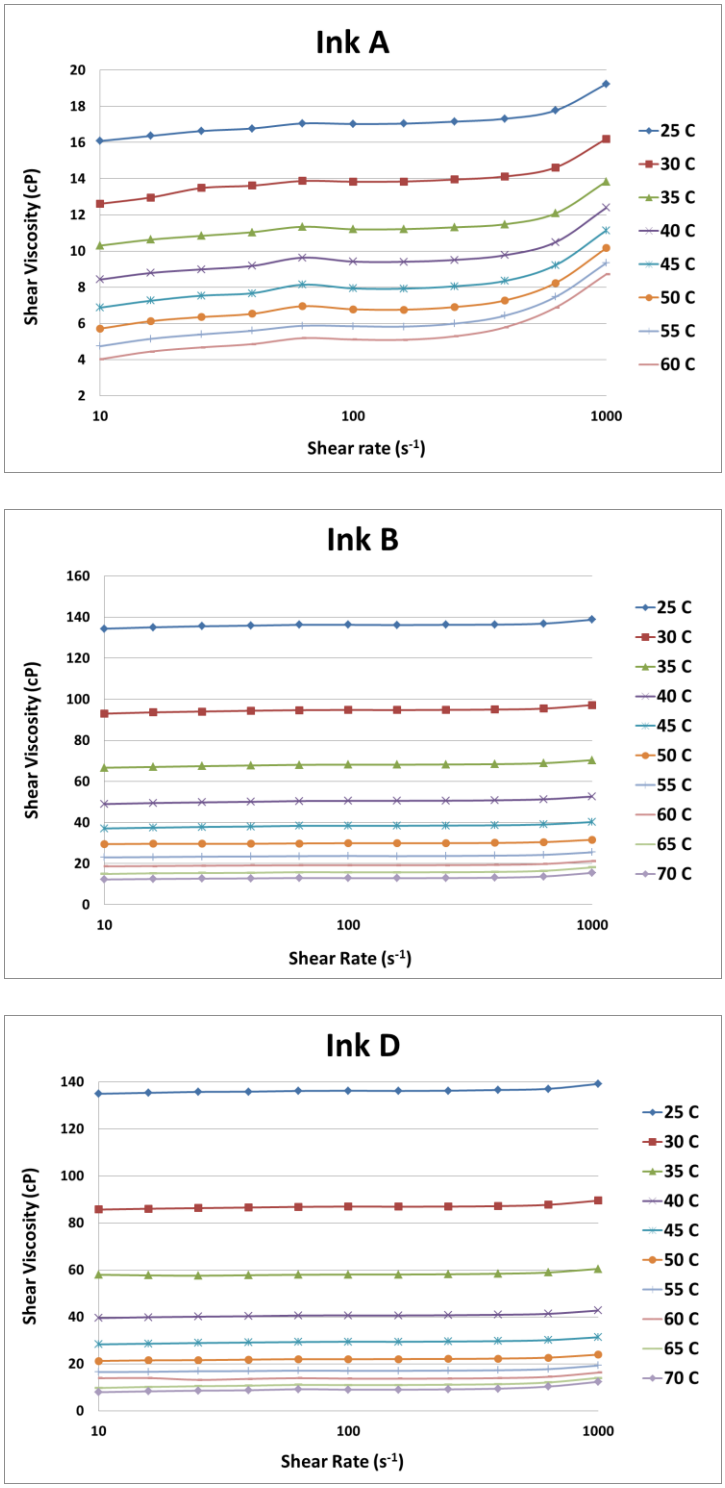

Figure 1: Viscosity data as a function of shear rate and temperature for inks A, B and D

Surface tension of inks A and D were measured directly at their printing temperature. But the printing temperature of ink B was 70ºC, higher than the maximum operating temperature of the Kruss DSA100S. However, as demonstrated in Figure 2, the variation of surface tension of ink B at temperatures above 45ºC is almost absent, approaching to 31 mN/m. Keeping in mind that surface tension influences Z but not significantly, this is the value which was considered as the surface tension of this ink at 70ºC. Pure monomer surface tension[22] was used for ink C, due to the same reason provided for viscosity.

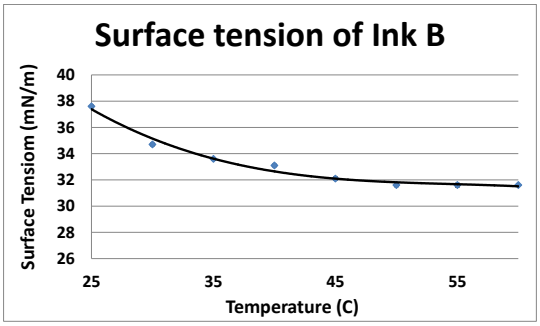

Figure 2: Surface tension of ink B. As shown, the viscosity value of the ink is approaching to 31 mN/m with temperature

The variation of density in liquids with temperature (lower than their boiling point) is insignificant. The addition of 1 % of a solid in such liquid also results in an irrelevant increment of density. So, in terms of Z parameter calculation, density values of pure monomers were used.

The printability parameter was calculated for each acrylate from the values obtained for viscosity and surface tension, the pure monomer density and a nozzle diameter of 21 µm. Results are shown in Table 1. These data demonstrate that inks A, B and D, displaying Z values of 3, 2.1 and 2 respectively, are printable and possess a stable droplet generation (Figure 3). However, ink C showed a Z parameter of 14.7. This value suggested ink C would not have stable droplet formation under normal conditions (waveform, voltage, jetting frequency). In Figure 4, the standard waveform for printing inks A, B and D is shown. Despite this Z value, ink C was printed with stable droplet generation using a complex waveform, designed for low viscosity inks (Figure 3 and 4). A summary table showing printing parameters such as printing temperature, voltage and jetting frequency are shown in Table 2.

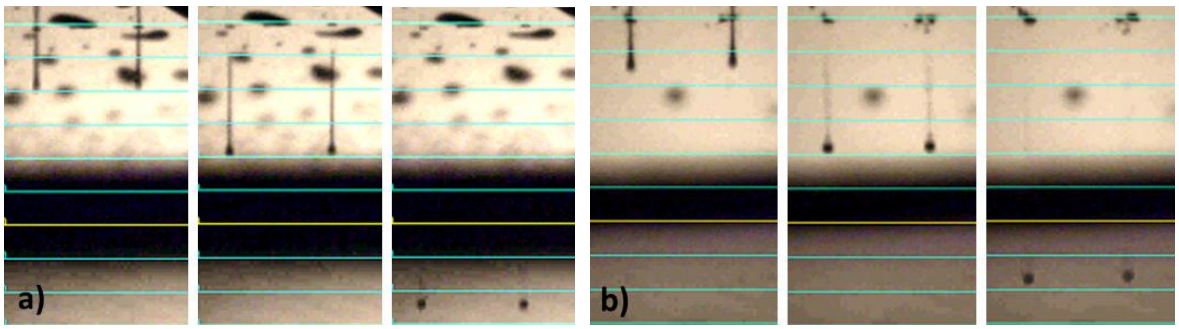

Figure 3: a) Sequence of droplet formation of ink A with standard waveform; b) Sequence of droplet formation of ink C with complex waveform

Inks	Printing Temperature (ºC)	Voltage (v)	Jetting Frequency (KHz)	Drop Spacing (µm)
A	40	19-20	1	25
B	70	19-21	6	30
C	25	18-19	1	-
D	60	20-22	8	25

Table 2: Printing parameters for each ink

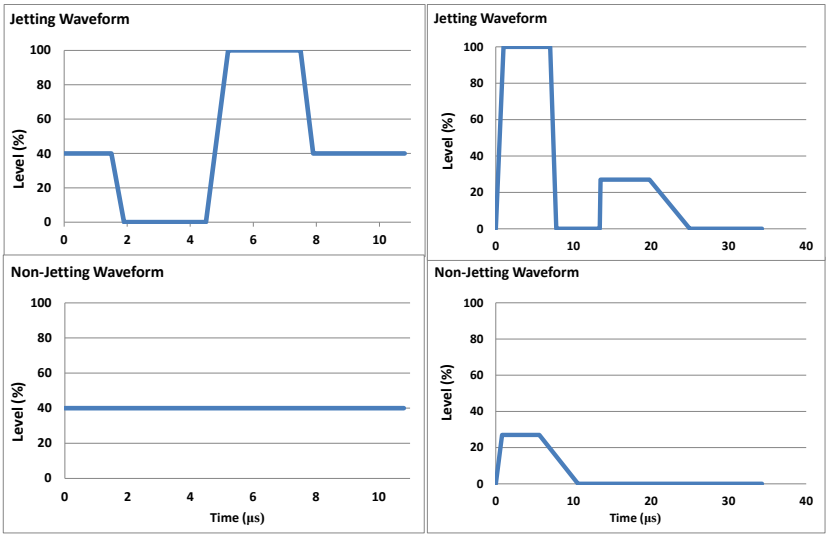

Figure 4: a) Standard waveform used for printing inks A, B and D. b) Complex waveform used for ink C

Once the printability parameters were optimised, the ideal drop spacing for a certain substrate had to be established to generate a 3D structure in order to minimise surface roughness. PET and glass were investigated as substrates; results displayed the same drop spacing could be used for both materials (Table 2).

Ink C was ejected with high stability using its corresponding printing parameters. Despite using the lowest jetting frequency possible, to increase the exposure time to UV light of the sample, the ink didn't cure and became a solid. However, 3D structures built with curable monomers are shown in Figure 5

Figure 5: 3D structures built with inks A [a)], ink B [b)] and ink D [c)]

Conclusions

Four inks from four different monomers with resistance to bacterial attachment have been developed using 1 % of DMPA as photoinitiator. Printability has been investigated via Z parameter. Printing temperature was set for each ink and the target physical properties (shear viscosity and surface tension) were measured. After calculating Z parameter, inks A, B and D, displaying Z values of 3, 2.1 and 2 respectively, showed to be printable. However, ink C displayed a Z value over the printable range. Nevertheless, the use of a complex waveform allowed this ink to be ejected with high stability. This led to build 3D structures using inks A, B and D. But, even though ink C was printable, it did not cure after jetting. For future work,

the modification of different variables for this ink will be investigated, such as UV exposure time or height or photoinitiator concentration, among others.

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