POLYMER PARTICLE FORMATION USING INKJET PRINTING

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Abstract

Exciting advances have been made in biomaterials research, through both relating material properties to cell response and discovery of new materials via high throughput screening. This area of research is still hindered though by the paucity of information on the physicochemical parameters governing the response of cells to a broad range of materials. Herein, a combinatorial library of biodegradable, photocrosslinkable and microparticle-forming polymers is generated by transforming a macro-performed pipetting experiment into a micro-sized piezoelectric inkjet printing. Physiochemical properties such as density, polymerization rate, surface tension, viscosity and solubility have been shown to be critical for successful single and multiple polymer structured microparticles. The vision is to mature this effort for applications that require biocompatibility such as drug delivery and cell carriers in regenerative medicine strategies to engineer cell functions.

1. Introduction

Single and multiple polymer structured microparticles provide a unique platform for a wide range of applications such as ultrasound contrast agent [1] as well as verification standards for instance for explosives trace detection instruments [2]. They have also been used for drug delivery [3] and tissue engineering [4]. However, microparticles are also useful in photonic bandgap materials [5], light diffusers in display panels and painting materials [6]. Conventionally, polymeric microparticles have been produced by a number of techniques such as spraying [7], phase separation [8,9], emulsion [10] and microfluidic based techniques [11]. The majority of these common methods mostly produce particles from alginate and polyesters like poly(lactide), poly(glycolide) and poly(lactide-co-glycolide) in a wide range of sizes: 5 to > 500 µm with spraying [12] and 0.05-1 µm with emulsion polymerization [11]. However, controlling the shape, average sphere diameter and size distribution are lacking with these techniques which limit the use of polymeric delivery vehicle in a wide field of application [12–14]. Moreover, in order to allow for practical application in a high throughput manner, the low production rates as well as the inability to readily access a library of materials need to be overcome.

A recent versatile manufacturing technique called inkjet printing has become an attractive process. It is capable of precisely depositing picolitre volumes of fluid in well-defined patterns without the need for masks [15,16]. As a non-contact patterning technology, inkjet printing has emerged as a tool for the fabrication of particles with precisely controlled and uniform size distributions [1,2,12] but also for printing cellular structures [17–20]. A few methods have been reported for the fabrication of microparticles with controlled sizes using an oil/water emulsion

solvent evaporation piezoelectric printing process [1,2,12]. So far the production of microspheres mostly involved solvent-based polymers, which limit the range of polymers that can be printed since these must be polymerized in advance.

The focus of this research lies on the extension of materials discovery, particularly from 2 to 3D. Despite some advances made through both relating material properties to cell response and discovery of new materials, rational material design of new materials is still hindered by the lack of knowledge on the physiochemical parameters. These parameters control the range of cellular responses required of modern devices. Recently, there have been a number of notable successes for discovery of novel biomaterials applying a high throughput screening approach [21,22]. For example, the identification of a new class of polymers resistant to bacterial attachment [23]. A series of materials which allow long-term renewal of pluripotent stem cells has also been reported on [24].

For this study a methodology for producing solid microparticles has been developed, using a combination of photocrosslinkable polymers and piezoelectric inkjet printing. In this report, the capability of transforming a macro-performed pipetting experiment into a micro-sized inkjet printing is presented in order to generate uniform polymeric particles. Furthermore, the difficulties in finding suitable combinations of photocrosslinkable inks with collecting fluids are highlighted to generate a library of microparticles with a wide range of chemistry and to predict the success rate for particle fabrication by analyzing the material properties.

2. Materials and methods

2.1. Materials

The photocurable polymerization solutions were based on acrylates/methacrylates most of which have been shown to be biocompatible and biodegradable [21,23,25]. For crosslinking in air and under reduced oxygen atmosphere, a photoinitiator (air: 3 wt%, reduced oxygen: 1 wt%) with and without an accelerator (3 wt%), respectively, were dissolved in the monomer. As collecting fluids, aqueous solutions with different polarity indices were used. Viscosities and surface tensions of the inks and collecting fluids were measured by a rotational viscometer (Malvern Kinenux Rheometer) and Krüss Drop Shape Analyzer DSA100, respectively.

2.2. Manufacture of polymer microparticles

Polymeric microspheres were prepared by using a piezoelectric inkjet printer (Dimatix DMP-2800, Fujifilm Dimatix Inc.) with typical drop sizes of 10 picolitres. The photocrosslinkable polymerization solution was loaded into a cartridge and then printed into a well plate filled with aqueous collecting fluids. The droplets were ejected with a single nozzle at the firing voltages and frequency of 20-25 V and 3 kHz, respectively. The actual jetting behavior of the fluid was viewed by using a built-in drop jetting observation system.

2.3. Optical and scanning electron microscopy

The microsphere diameter and degree of monodispersity was examined using a Smart Imaging System (IMSTAR S.A.) optical microscope with 40x objective lens. The samples were imaged in the well plate and the mean diameter of selected particles was measured using the software IMSTAR array. The shape and surface morphology of the printed polymeric particles were assessed using a scanning electron microscope (SEM) at 10 kV accelerating voltage. The particles were placed onto the sample holder by adding a drop of suspension. Prior to SEM analysis, the samples were sputter-coated for 4 minutes at 25 mA with a thin gold layer in an argon atmosphere.

2.4. Particle size distribution

The particle size distributions were measured on a CPS disc centrifuge equipped with a 405 nm light source. Centrifugal sedimentation within an optically clear spinning disc is applied for the measurement. The spinning disc was filled with a sucrose density gradient to stabilize the sedimentation of particles and dodecane was used to prevent gradient evaporation. Aqueous suspensions containing the microspheres (0.1 ml each) were sized after calibrating the instrument with 1.27 micron PVC dispersed in water prior to each test.

2.5. Focused ion beam measurement

In order to analyze whether the particles are hollow or solid in the interior, a focused ion beam measurement (FIB) was performed using a Zeiss NVision 40 CrossBeam machine equipped with a Ga source. The sectioning was conducted at 30 kV with a probe current of 1.5 nA. The particles were placed onto the sample holder and sputter-coated for 4 minutes at 25 mA with a thin gold layer in an argon atmosphere prior to FIB measurement.

3. Results and discussion

3.1. Manual pipetting experiments

Particles of 10 photocrosslinkable solutions were obtained mostly in solely one collecting fluid when pipetted a small volume (5 µl) into a well plate under air. By repeating the experiment under reduced oxygen atmosphere, more inks succeeded in particulate generation. The reason is that oxygen works as an inhibitor for the polymerization by quenching the free radicals. Even after changing to reduced oxygen atmosphere, some polymerization solutions did still not lead to particles independent on the polarity of the collecting fluid. Thin polymeric films, hemispherical shapes stuck to the bottom of the well and also dissolution of the ink in the collecting fluid was observed. This inability to form particles is likely due to insufficient polymerization rates. Furthermore, drop formation into a fluid requires understanding of the concepts of surface tension, viscosity and solubility. The solubility parameter plays a role when the ink drop remains in a liquid state and has not crosslinked before touching the collecting fluids. In this case, the ink will dissolve in a solvent with a similar chemical structure to itself.

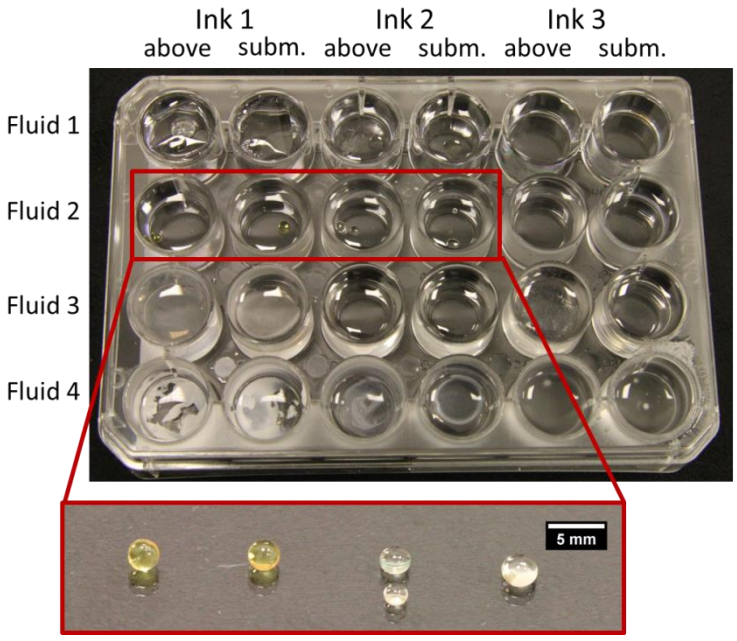


Figure 1 Image taken after pipetting 5 µl of different polymerization solutions above and submerged into 4 different collecting fluids with varied polarity. The zoomed image shows the particles obtained from 2 different inks in solely one collecting fluid.

The pipetting of the polymerization solutions was conducted from above the collecting fluid and with the pipette tip already submerged into the fluid as is shown in Figure 1. These two configurations were chosen to analyze the impact of the liquid-gas interface on the drop shape preservation. The results showed that there is no significant difference obtained between pipetting 5 µl of ink above and submerged. However, the ink 2 dispensed from above resulted in two particles. This may happened due to separation at the interface. In order to gain a better understanding of the factors influencing the generation of drops, videos of pipetting a polymerization solution into the 3 most promising collecting fluids and air (reference) were recorded utilizing a high speed camera.

Table 1 illustrates that droplets formed easily by dispensing into air and also into fluid 2 both from above and below the liquid-gas interface. In case of pipetting above fluid 2, a stream of liquid was "injected" into the bulk liquid, narrowed its cross-section and formed a drop below the surface. In contrast, a generated drop sinks without larger shape modifications in case of submerged pipetting. By utilizing fluid 1 and fluid 4, spherical drops could not be produced. On the one hand spreading of the ink occurred at the surface with fluid 1. On the other hand sinking of the ink without retaining the shape was observed with fluid 4.

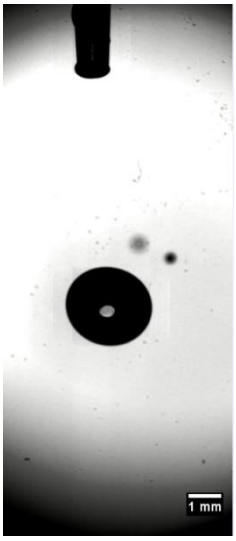
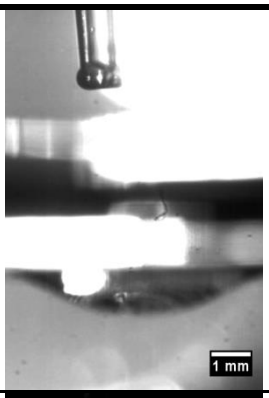
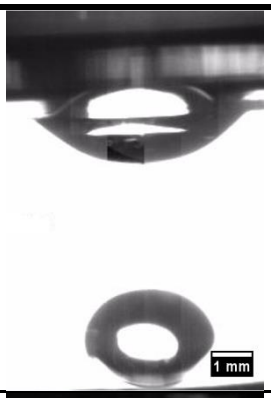
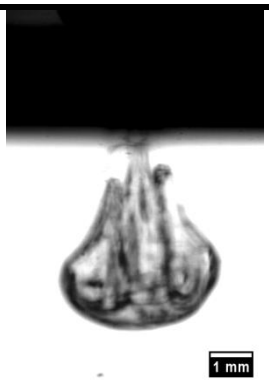
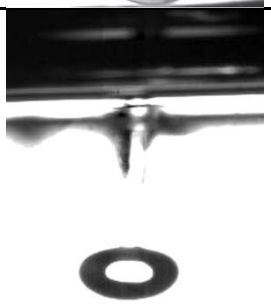
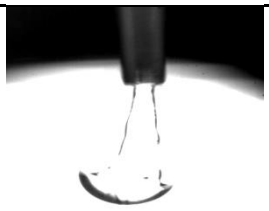
	Air	Fluid 1	Fluid 2	Fluid 4
Above collecting fluid				
Submerged pipette tip				

Table 1 Sequences of videos recording 5 µl of an acrylate-based ink pipetted above and below the collecting fluids. Pipetting the ink into air served as reference.

3.2. Formation of solid microparticles

Promising photocrosslinkable inks obtained from the pipetting experiment were inkjet printed into the 4 collecting fluids. Microspheres of an acrylate-based ink with a diameter in the range of 8.5 – 23.5 microns were only obtained in collecting fluid 2. In case of the other fluids, polymer clumps/agglomeration were found (Table 2).

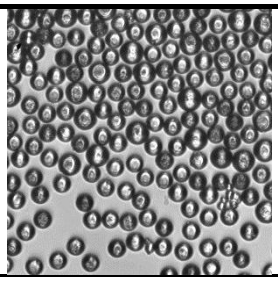
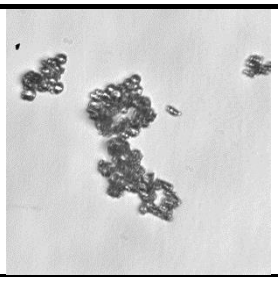
	Fluid 2	Other Fluids
Optical micrographs (40x magnification)		

Table 2 Bright field micrographs showing printed particles in fluid 2 and polymer clumps in other fluids. The images were taken at a 40x magnification.

The inkjet printed particles were uniform and exhibited a smooth surface containing some pores (Figure 2). A few smaller microspheres were also visible which are likely formed by the break-up of the drops while touching the liquid-gas interface. Satellites are usually significantly

smaller than the particles and hence, they can be excluded since such small particles were not observed in the printing experiment.

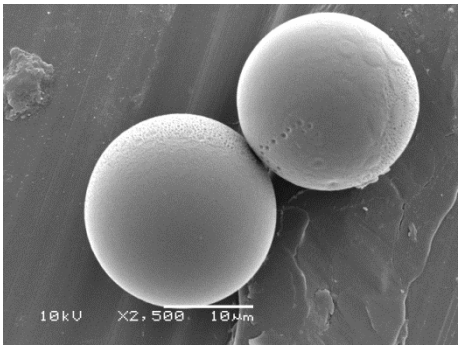


Figure 2 SEM picture of uniform microspheres obtained by jetting a polymerization solution into fluid 2.

The narrow size distribution as observed with the optical microscope and SEM was confirmed with CPS disc centrifuge. An average diameter of 17.37 µm was calculated with a coefficient of variance of 33.24% (Figure 3).

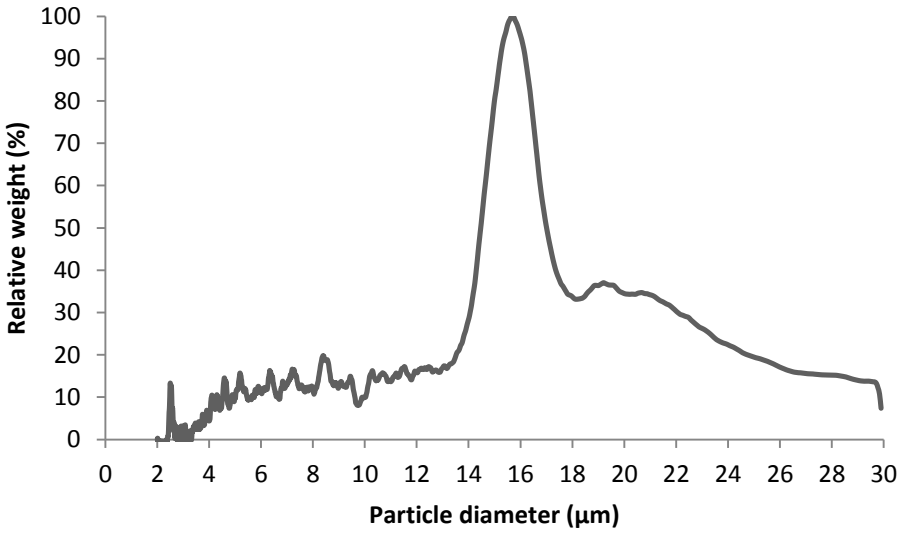


Figure 3 Particle size distribution of microspheres was measured using centrifugal sedimentation stabilized by a sucrose density gradient.

The particle interior was analyzed by slicing the microsphere using FIB. As seen from Figure 4, the inkjet printed particles were solid throughout. Therefore, the results indicate that neither air nor collecting fluid was trapped inside the particles.

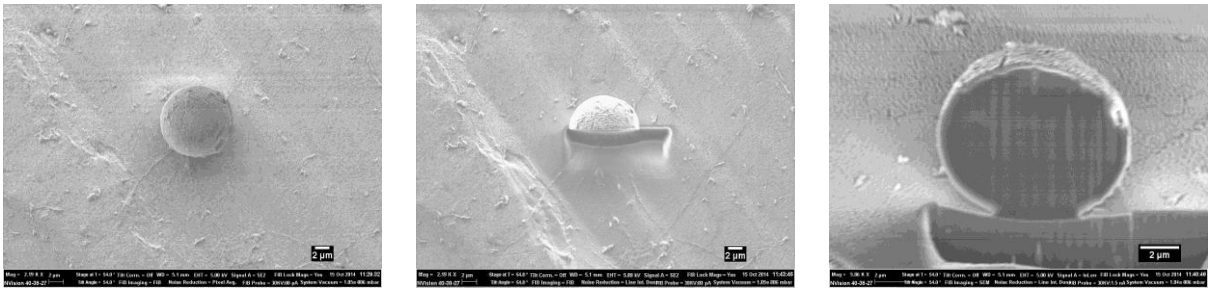


Figure 4 FIB images of an inkjet printed particle before (left) and after sectioning (middle: top view, right: side view).

The results of this printing study reflect the phenomena found in the manual pipetting experiment to a large extent. Reducing the volume from pipetting (µl) to printing (pl), complicates the understanding of parameters affecting the particle formation. Besides density, polymerization rate, viscosity and solubility, factors related to the surface such as surface energy/tension, surface charge become more important the smaller the volume, consequently the smaller the particle.

4. Conclusions

We have developed a methodology capable of producing monodispere microparticles using piezoelectric inkjet printing and photocrosslinkable polymers. The technique has a potential to overcome problems of conventional technologies, like the restriction to generate a large number of various polymers, the lack of controlling the shape, size and size distribution as well as the low-throughput production. The capability of transforming a macro-performed pipetting experiment into a micro-sized inkjet printing with a relative high reliability was demonstrated. Furthermore, physiochemical properties such as density, polymerization rate, surface tension, viscosity and solubility have been shown to be critical for successful microsphere formation. However, there are still a few unknown factors which restrict a complete understanding of the particulate formation process. Therefore, further interdependency analysis will be performed to be able of finding suitable combinations of polymerization solutions and collecting fluids without applying trial and error approach. Advancing this research is attractive in order to generate a library of microparticle-forming polymers with a wide range of chemistries for applications that require biocompatibility.

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