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# MICROVALVE BIOPRINTING OF CELLULAR DROPLETS WITH HIGH RESOLUTION AND CONSISTENCY

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**ABSTRACT:** Cellular printing via bioprinting approaches holds tremendous potential in fabrication of tissues/organs. One of the attractive traits of bioprinting systems include precise control over the spatial deposition of multiple types of cells. Notably, cell sedimentation during printing process poses a huge challenge and the ability to maintain printing consistency over time is required to realize potential scale-up of bioprinting processes. To achieve successful controlled cellular deposition, it is ideal to ensure an improved printing output consistency and high dispensing efficiency at high printing resolution over time. In this work, we demonstrated that the use of polyvinylpyrrolidone 360KDa (PVP360) polymer in printing solution enhances the printing resolution and output consistency over a period of 30 minutes. We also determined the minimum cellular density required to achieve a high dispensing efficiency of > 95%.

**KEYWORDS:** 3D printing; bioprinting; rapid prototyping; additive manufacturing; microvalve-printing

## 1. INTRODUCTION

The native tissues have unique patterns created by natural compartmentalization of different types of cells that are positioned relative to each other at high degree of specificity (W. L. Ng, Yeong, & Naing, 2015; Tobin, 2006). The ability to control spatial deposition of multiple types of cells in an accurate and repeatable manner is of great interest to a variety of biological applications. These potential applications have spurred the development of variety of technologies encompassing reliable cell handling and positioning capabilities. 3D bioprinting has emerged as a leading paradigm for directed cell deposition (Murphy & Atala, 2014), which include laser-assisted, extrusion-based, inkjet-based and microvalve-based bioprinting systems (J. M. Lee & Yeong, 2014; W. L. Ng, Yeong, & Naing, 2016; W. L. Ng, Yeong, W. Y. & Naing, M. W., 2014; Tan, 2014).

Among the above-mentioned systems, drop-on-demand (DOD) printing (inkjet- and microvalve-based) is a promising approach for controlled deposition of cellular droplets. Inkjet printing has been shown as a viable technique in scaffold fabrication (Chua, Yeong, & Leong, 2005; Yeong, Chua, Leong, Chandrasekaran, & Lee, 2005). Some inherent limitations of such DOD printing systems include narrow range of printing viscosities (1-10 mPa.s), low printable cellular density (< 3millions cells/ml) and possible clogging issues (W. Lee et al., 2009). The inkjet-based system has a relatively higher printing resolution due to its smaller orifice (< 100  $\mu\text{m}$ ) and it has a more complex set-up consisting of reservoir, tubing and nozzle. This increases the probability of clogging issues due to sedimentation effects during the printing process. In contrast, the microvalve-based printing system has a relatively simple set-up consisting of a reservoir and

nozzle. The operation of the microvalve-based system is based on a combination of both pneumatic pressure and valve opening time at the nozzle; droplet deposition is controlled by the rheological properties of the fluid as well as the geometrical properties of the nozzle tip.

Pioneering works have been conducted to demonstrate the feasibility of controlled cellular deposition (Nakamura et al., 2005; Roth et al., 2004). Subsequent works focus on printing of cellular droplets to form tissues (W. Lee et al., 2009; Moon et al., 2009). Nevertheless, one of the main challenges in bioprinting process is to mitigate cellular aggregation and ensure consistent cellular output. It has been shown that gravitational forces acting on the cells during the printing process can lead to cell sedimentation and changes the uniformity of cell suspension over time. As such, it is important to maintain printing consistency over time in order to realize the potential scale-up of bioprinting processes.

The sedimentation effect on bioprinter output was then characterized in a study (Pepper, Seshadri, Burg, Burg, & Groff, 2012), highlighting the problem of inconsistency printing output over time. Although attempts to mitigate the sedimentation effects have been conducted (Chahal, Ahmadi, & Cheung, 2012; het Panhuis, 2013), the dispensing efficiency (number of printed droplets containing at least a single cell) in these studies were low (< 75%).

In this work, polyvinylpyrrolidone 360KDa (PVP360), a water-soluble polymer, commonly used in cell separation applications was utilized to mitigate the sedimentation effects in the microvalve-based dispensing system. We evaluated the microvalve bioprinting output in terms of printing resolution and printing consistency.

## **2. MATERIALS AND METHODS**

### **2.1. Cell culture**

Neonatal human foreskin fibroblasts (HFF-1 from ATCC® SCRC-1041™) were used in this study. The cell line was cultured in a HERAcCell 150i cell incubator (Thermo Scientific) at 37 °C in 5% CO<sub>2</sub> using ATCC-formulated Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 15% fetal bovine serum (HyClone™ from GE Healthcare). The adherent fibroblast cells were harvested using 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA) (Invitrogen) at 90% confluency.

### **2.2. Preparation of printing cellular solution**

Phosphate buffered saline (PBS) solution (pH 7.4 at 0.01 M) was sterile-filtered before use. A stock PVP solution of 5% w/v in 1x PBS solution was prepared by mechanical agitation. The suspension consisting of 0.25, 0.50, 0.75 and 1.00 million cells/ml and desired PVP360 concentration (0-3% w/v) were prepared by diluting initial cell concentration and stock PVP solution (5% w/v).

### **2.3. Viscosity Measurements**

The rheological properties of cell suspensions with varying concentrations of PVP360 (0–3% w/v) were evaluated using the Discovery hybrid rheometer (TA instruments, USA). The viscosities of

cell suspensions (PVP with varying % w/v and cellular density) were evaluated for shear rates ranging from 0.1 to 100 s<sup>-1</sup> at a constant temperature of 27 °C (printing temperature).

#### 2.4. Microvalve-based dispensing of cellular droplets

A 3-D bioprinter, Biofactory<sup>®</sup> (regenHU Ltd., Switzerland), was used for printing of cell suspensions. The microvalve-based printhead has an inner nozzle diameter of 100 μm. Pre-defined patterns (5 sets of 3x3 dots array) were input into BioCAD (regenHU Ltd., Switzerland). The number of cells within each printed droplets in our printing system can be controlled by the pneumatic pressure, valve opening time (correlate to droplet volume) and cellular density within the suspension. The cells were stained with calcein AM (Molecular Probes<sup>®</sup> Live/Dead staining kits, Life-Technologies) according to the manufacturer's manual prior to printing. We evaluated the bioprinter output in terms of printing resolution and consistency.

### 3. RESULTS AND DISCUSSION

#### 3.1. Viscosity Measurements

The viscosity of printing solution is known to influence printing resolution (Malda et al., 2013) and cell sedimentation (Wang & Belovich, 2010). The microvalve-based print-head used in this study is only capable of ejecting fluids within a narrow viscosity range of 1 – 8 mPa.s. Figure 1 shows that printing solutions up to 2.5% w/v PVP remain printable at shear rates ranging from 10<sup>1</sup> to 10<sup>3</sup> s<sup>-1</sup>. The printing solutions generally exhibited shear-thinning behaviors with decreasing viscosities at increased shear rates and plateau as shear rates are further increased beyond 10<sup>1</sup> s<sup>-1</sup>. The generated shear rates at the nozzle of microvalve print-head is calculated to be in the range of 10<sup>5</sup> s<sup>-1</sup>. The measurements offer adequate representation of the viscosity generated at the printing nozzle and the highest printable PVP solution of 2.5% was used in the subsequent experiments.

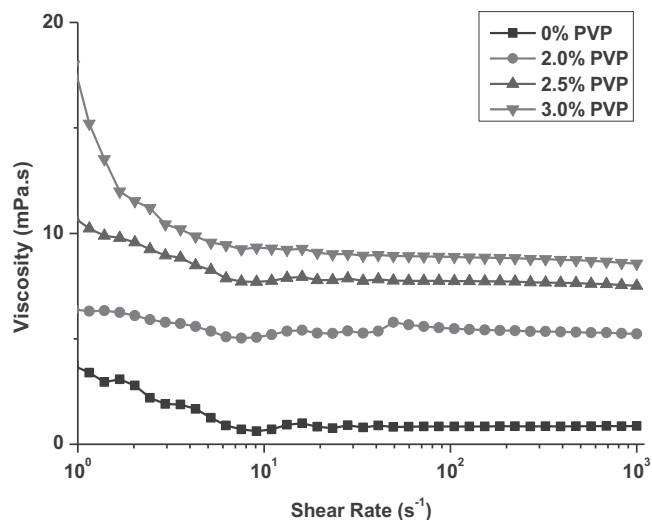


Figure 1: Viscosities of varying PVP (% w/v) suspensions as a function of shear rate (s<sup>-1</sup>)

### 3.2. Effect of PVP on printing resolution

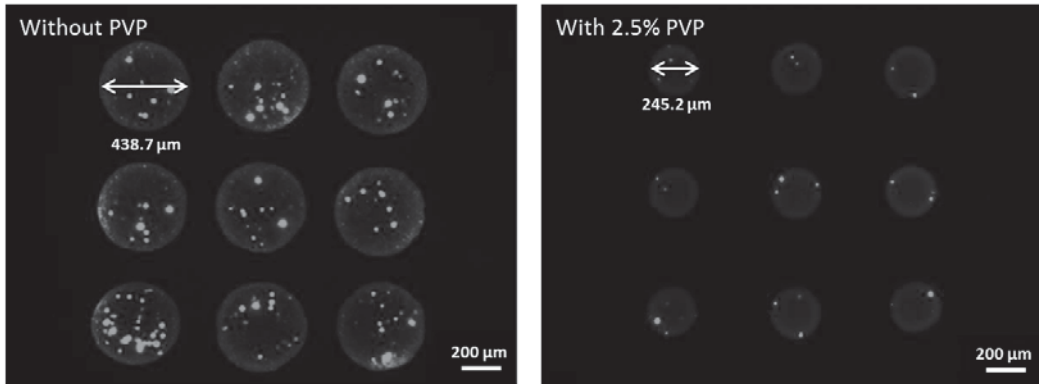


Figure 2. Representative images of printed cellular droplets (Left) 0% PVP and (Right) 2.5% PVP cell suspension printed onto glass slide.

The printing resolution of the cellular droplets remained relatively constant over the printing duration of 30 minutes. Upon addition of 2.5% PVP to the printing solution, the resolution of cellular droplets decreases from  $441.3 \pm 8.2 \mu\text{m}$  to  $254.4 \pm 7.8 \mu\text{m}$  (42.4% improvement in printing resolution) and the estimated volume per droplet decreases from 22.5 nL to 4.3 nL (80.9% reduction in volume) as shown in Figure 2.

### 3.3. Effect of PVP on printing consistency

We evaluate the sedimentation effect by monitoring the dispensing consistency over a period of 30 minutes. The examination was conducted by staining the cells with calcein AM prior to printing for easy identification of cells. In the absence of PVP polymer as shown in Figure 3, the cellular output gradually increased and peaked at 20 mins and then began to decrease regardless of the cellular density. The observations are corroborated by another study (Pepper et al., 2012) which also reported a similar trend in printing output. The addition of 2.5% w/v PVP to the printing solution increases both fluid density and viscosity, which in turn reduces sedimentation velocities. It was observed cellular number in the PVP droplets is relatively lower and more consistent and it gradually increases over a period of 30 minutes.

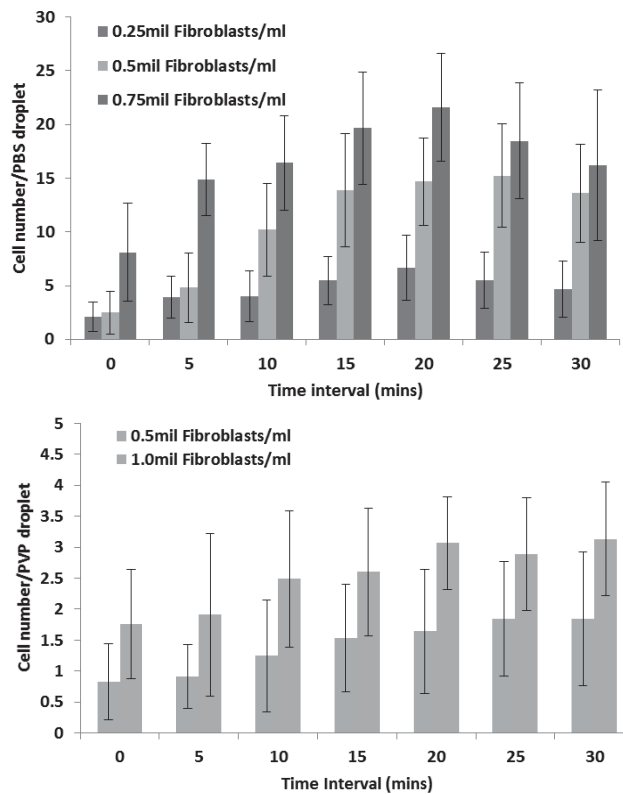


Figure 3. Cell count per cellular droplet (n=45) (Top) 0% PVP and (Bottom) 2.5% PVP cell suspension printed onto glass slide over a period of 30 minutes. The printed cellular droplets show 42.4% improvement in printing resolution upon addition of 2.5% PVP.

#### 4. CONCLUSION

To achieve successful controlled cellular deposition, it is imperative to acquire an improved printing output consistency and good dispensing efficiency at high printing resolution over time. In this work, we have demonstrated that the use of PVP solution enhances the printing resolution of cellular droplets (42.4% improvement from  $441.3 \pm 8.2 \mu\text{m}$  to  $254.4 \pm 7.8 \mu\text{m}$ ) and improves printing consistency over a period of 30 minutes. Furthermore, a minimum cellular density of 1mil cells/ml is also required to achieve a high dispensing efficiency of > 95% throughout a printing duration of 30 minutes.

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