Optimizing Alginate Microbead Formation using Handheld Bioprinting for Cell-Friendly Encapsulation

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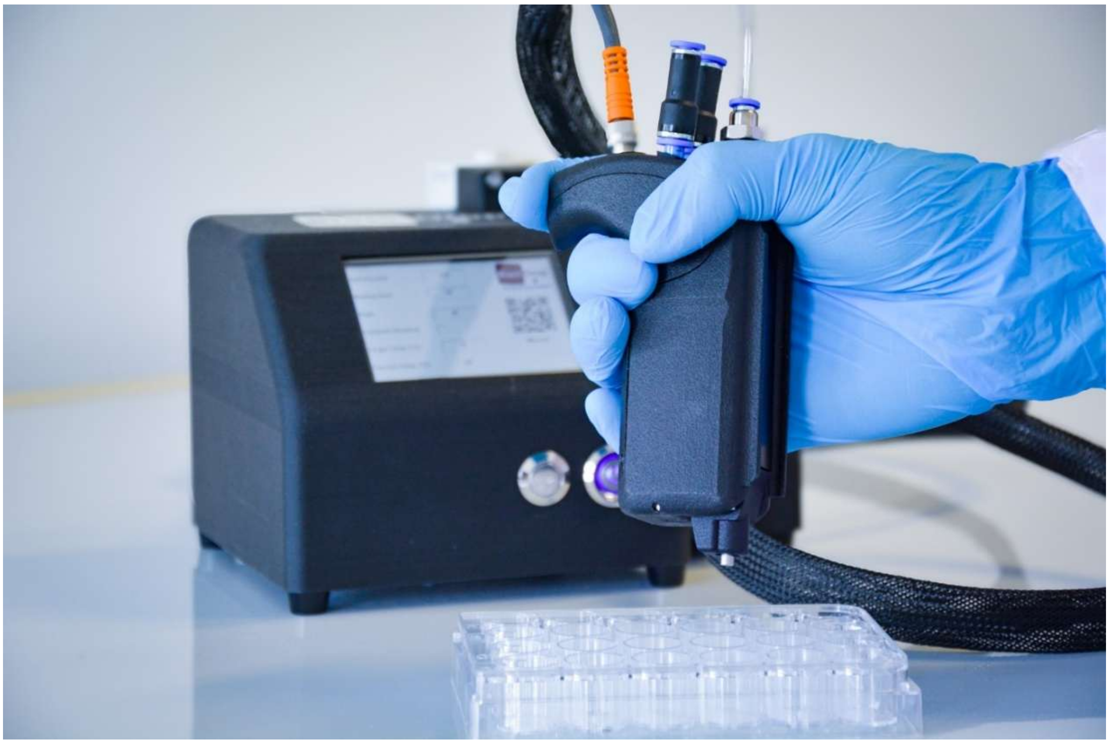


Abstract

This study investigates the optimization of alginate microbead fabrication using the Black Drop Dropgun handheld bioprinting station, focusing on key parameters for generating uniform and viable microenvironments for cell or drug encapsulation. We explored the effects of pneumatic pressure, opening time, waiting time between drops, nozzle-to-solution distance, and printhead movement on bead diameter and roundness. It became clear that pressures above 0.3 bar prevent droplet formation. An ideal nozzle-to-solution distance of 2 cm, with a 90° impact angle, was established. Tests comparing moving versus non-moving printheads revealed that a stationary printhead is sufficient as long as distance and angle are optimized. Crucially, while increased pressure negatively impacted bead shape, it did not significantly alter diameter, allowing independent control of these characteristics. Bead diameter was precisely tunable by adjusting the microvalve opening time. ImageJ analysis confirmed these results. This parameter study provides a reliable methodology for producing high-quality, cell-friendly alginate microbeads suitable for various biomedical applications that can also be easily transferred into a Regenate Bioprinter.

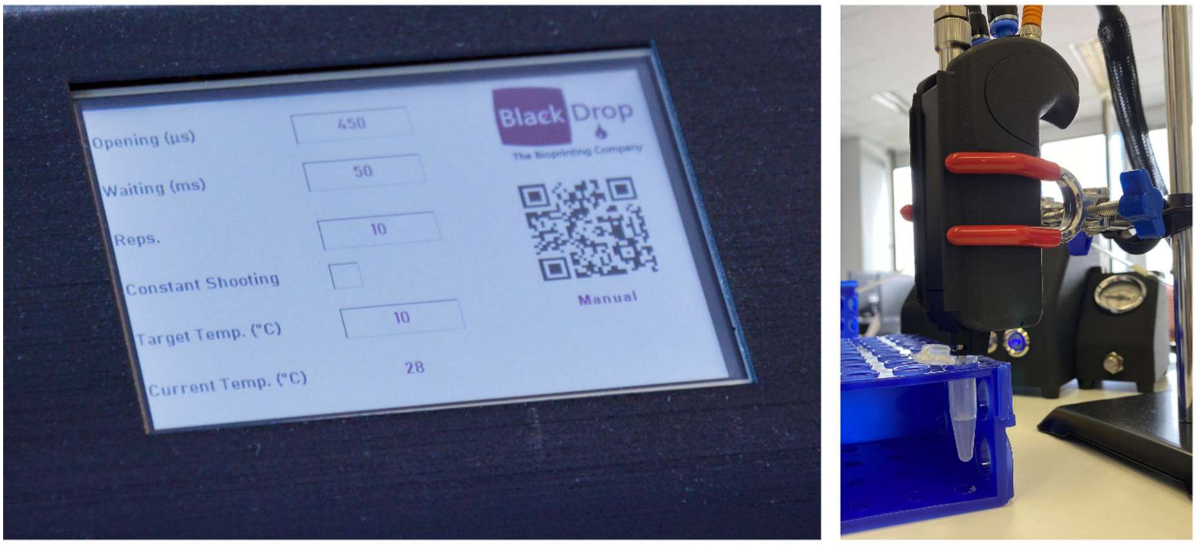
Introduction

Bioprinting has emerged as a transformative technology in tissue engineering and regenerative medicine, enabling the precise fabrication of constructs with controlled architectures. Among various bioprinting techniques, Drop-on-Demand (DoD) systems offer exceptional control over droplet size and placement, making them ideal for microencapsulation applications. Alginate, a biocompatible and non-toxic polysaccharide, is widely favored as a bioink due to its rapid ionic crosslinking capabilities in the presence of divalent cations like calcium. The ability to precisely control the size and shape of alginate microbeads is paramount for applications ranging from drug delivery research to cell encapsulation, where uniformity and cell viability are critical. This study aims to systematically investigate and optimize the printing parameters for a DoD bioprinting process to produce alginate microbeads with high reproducibility and desired characteristics, specifically focusing on factors influencing bead diameter and roundness for cell-friendly encapsulation.

Figure 1: The Black Drop Dropgun handheld bioprinting station consists of an ergonomic handheld that allows precise bioink deposition pressing a trigger button. Dispension parameters can be conveniently set via the touch-screen on the controlling station while the pressure of the sterile-filtered air supply can be adjusted using the manometer.

Materials and Methods

The Black Drop Dropgun handheld bioprinting station was used with a DoD printhead, equipped with a 300 µm microvalve. All experiments were done at 21 °C room temperature. The ergonomic handheld was easily mounted in a standard laboratory stand, allowing for a complete experimental setup in under 5 minutes. Blue Drop Alginate (2%) was used as the bioink, and 500 µl of a CaCl2-containing crosslinking solution was placed in a 1 ml centrifuge tube to collect the dispensed droplets. Prior to parameter studies, an initial cleaning of the printhead was performed by dispensing 100 drops into a discard petri dish to ensure consistent droplet formation. Shooting was conveniently triggered by the button on the housing. For each experimental condition, exactly 50 drops were dispensed to ensure statistical relevance. The parameters varied included dispense pressure, microvalve opening time, and waiting time between consecutive drops. The distance between the nozzle exit and the surface of the crosslinking solution was also a critical parameter, with a final optimized distance of 2 cm established to achieve an ideal 90° impact angle for droplet formation. Evaluation of the bead size and shapes was done using a confocal microscope and ImageJ particle analysis.



Opening (µs) 450
Waiting (ms) 50
Reps. 10
Constant Shooting ☐
Target Temp. (°C) 10
Current Temp. (°C) 28

Figure 2: The touch screen (left) shows all parameters that can be controlled besides pneumatic air pressure. The DoD handheld was easily mounted in a standard laboratory stand ensuring a reproducible experimental setup (right) for dispensing alginate drops into a crosslinking solution.

Results

The systematic parameter study revealed several key findings regarding the fabrication of alginate microbeads. It was observed that maintaining pneumatic pressure within a specific range was critical for droplet formation; specifically, pressures above 0.3 bar consistently failed to produce drops. The nozzle-to-solution distance significantly influenced droplet integrity, with an optimal distance of 2 cm ensuring spherical bead formation and a targeted 90° impact angle. Figure 3 illustrates that the average diameter of the microbeads was directly and easily adjustable by varying the microvalve opening time, providing precise control over bead size. At 0.2 bar, increasing the opening time from 1000 µs to 4500 µs resulted in an increase in average diameter of approximately 40 %. Similar trends were observed at 0.3 bar, although with slightly larger diameters for comparable opening times. Notably, an increase in dispense pressure, while influencing the shape of the beads, did not significantly alter their average diameter. For instance, at 1500 µs opening time, the average diameter was approximately 1.2 mm at 0.2 bar and 1.25 mm at 0.3 bar, indicating a relatively stable diameter despite pressure changes. This characteristic is particularly advantageous for cell encapsulation applications, as it allows for independent optimization of bead shape without compromising the intended size. Figure 4 demonstrates that average roundness tended to decrease with increasing pressure, particularly noticeable at 0.3 bar compared to 0.2 bar, where roundness values remained closer to 0.9 for most opening times.

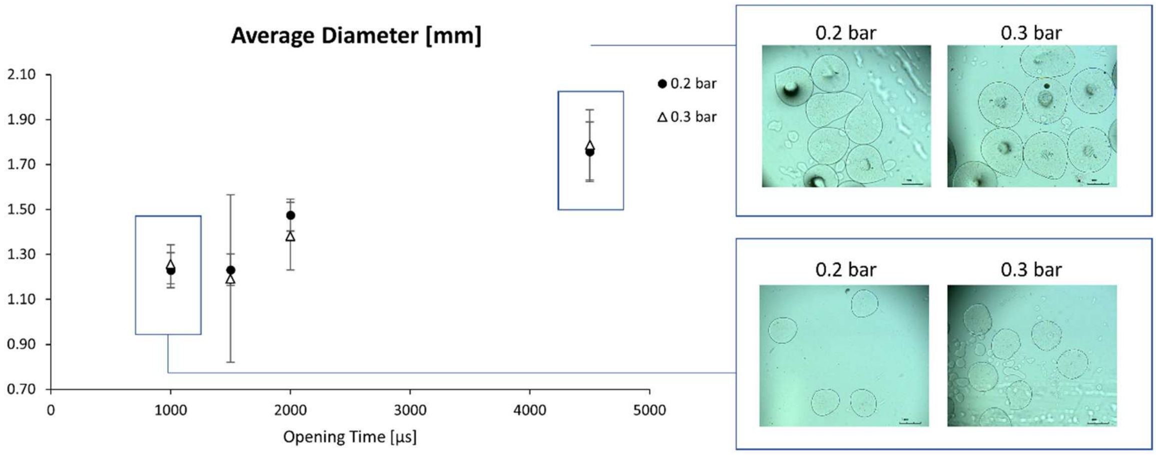


Figure 3: Average diameter of alginate microbeads as a function of opening time at 0.2 bar and 0.3 bar pressure. Error bars represent standard deviation. Diameter is shown to be adjustable by opening time and independent of applied pressure.

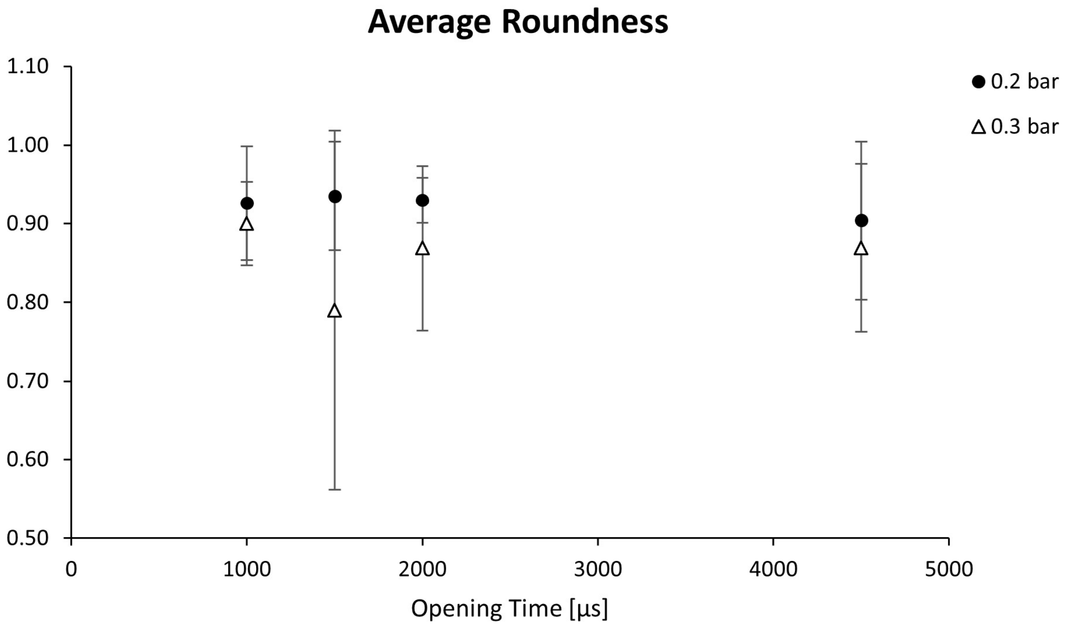


Figure 4: Average roundness of alginate microbeads as a function of opening time at 0.2 bar and 0.3 bar pressure. Error bars represent standard deviation. Higher pressures negatively impact roundness.

Discussion

The findings from this study underscore the critical role of precisely controlled parameters in Drop-on-Demand bioprinting for successful alginate microbead fabrication. The observed pressure threshold of 0.3 bar highlights the importance of optimizing fluidic dynamics to overcome surface tension and achieve consistent droplet detachment. The ideal nozzle-to-solution distance of 2 cm, aiming for a 90° impact angle, is crucial for minimizing droplet deformation upon impact with the crosslinking solution, thereby ensuring higher roundness and structural integrity of the resulting microbeads. A significant discovery was the ability to decouple bead diameter control from pressure-induced shape changes. The consistent diameter across varying pressures, particularly within the operational range (0.2-0.3 bar), indicates a robust and cell-friendly process. This allows researchers to fine-tune the opening time to achieve desired bead sizes for specific applications, such as cell encapsulation, while knowing that slight variations in pressure, perhaps due to system fluctuations or bioink rheology, will not drastically alter the cell's microenvironment size. Conversely, the shape of the beads, as quantified by the roundness, was more sensitive to pressure increases. This suggests that for applications demanding highly spherical beads, maintaining lower pressures within the optimal range is preferable. The ease of setup and mounting in a standard lab stand further enhances the practicality and accessibility of this DoD bioprinting system for routine laboratory use and rapid prototyping of biomaterials.

Conclusion

This comprehensive study successfully optimized key parameters for the fabrication of alginate microbeads using a user-friendly Drop-on-Demand bioprinting system. We identified critical operational limits for pressure, established an optimal nozzle-to-solution distance of 2 cm for spherical bead formation, and demonstrated the independent control of bead diameter through opening time adjustments. The remarkable finding that pressure primarily influences bead shape rather than diameter offers a cell-friendly advantage, enabling precise size control for encapsulated cells. This optimized methodology provides a robust and accessible platform for producing high-quality alginate microbeads, paving the way for advanced applications in cell encapsulation, drug delivery, and regenerative medicine, where controlled microenvironments are paramount.