Deformable beads on Nadia Innovate

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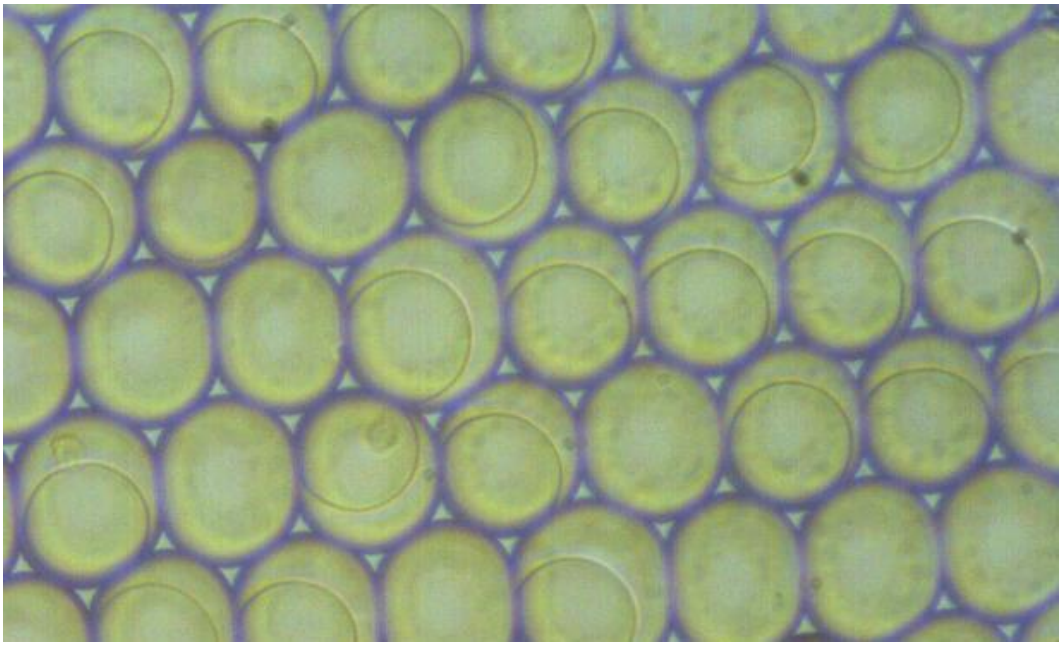

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Introduction

Recent advances in single cell research have led to the development of high-throughput single-cell RNA-seq (scRNA-seq), an application that allows transcriptome data from thousands of cells to be analysed in parallel at the single cell level. To make this technology more widely accessible, Dolomite Bio has developed the Nadia instrument range consisting of the Nadia Instrument, an automated, microfluidic droplet-based platform, and the Nadia Innovate, an open configurable system for rapid protocol development.

Currently, high throughput scRNA-seq on the Nadia Instrument is based on the "Drop-seq" method as described by Macosko *et al.* (1), which uses non-deformable solid beads. This approach enables fast encapsulation. However, beads must be flowed at a concentration that only allows capture of ~10% of cells in order to avoid bead doublet formation or blockages during a run, therefore rendering this approach less suitable for use with limited amounts of cell materials or rare cell detection.

In contrast, methods such as "inDrop" as described by Klein *et al.* (2) use deformable or "squishy" beads that can be encapsulated at a higher rate due to their microfluidic properties, therefore enabling an over 70% cell capture rate. This feature is essential when working with rarer cell populations.

This application note describes preliminary results on the use of deformable beads on Dolomite Bio's development platform, the Nadia Innovate.

Material and Methods

The possibility of developing a DropSeq-equivalent protocol on Nadia based on the use of deformable beads for improved cell capture rates was investigated by optimising parameters such as bead flowability, bead loading and pressure settings for stable monodisperse droplet production. The optimisation process was performed on the Nadia Innovate with use of commercially available deformable beads (inDrop™ Training Kit, 1CellBio).

Figure 1

Sample/ bead preparation and loading. Testing was performed with 70 µm non-barcoded deformable beads (inDrop™ Training Kit, 1CellBio). Deformable beads were spun down at 1,000 x g for 2 min, and approximately 1/3 of the supernatant was removed to concentrate bead suspension to 2/3 of its initial volume. Cells were suspended in cell suspension buffer (as described by Macosko *et al.* (1)) at a concentration of 300 cells/ µl. 100 µl of concentrated deformable beads and cell suspension were encapsulated on the Nadia Innovate together with 3 ml of BioRad oil as the carrier phase.

Results

To ensure ideal bead loading as well as cell and bead flowability conditions for DropSeq on Nadia, parameters such as pressure tables and stirrer speeds had previously been optimized for use with non-deformable beads resulting in a capture rate of ~10% of cells.

Due to the microfluidic properties of deformable beads significantly higher cell capture rates (>70%) can be obtained as shown by Klein *et al.* (2). To achieve similar values when using deformable beads on Nadia the following adjustments were found to be required:

- **Bead Stirrer Speed:** The bead stirrer speed was adjusted to 25 rpm as settling of beads is not expected to occur as fast as with non-deformable beads. Moreover, the hydrogel nature of deformable beads might render these beads more susceptible to breakage during stirring.
- **Pressure Tables:** Pressure tables were adjusted until stable droplet formation occurred at the microfluidic junction of the chip, and both cells and beads were mixed at equal volumes at the chip junction (Figure 2 A).
    - Beads: 100 mbar
    - Cells: 60 – 70 mbar
    - Oil: 260 mbar
- **Droplet Formation Speed:** 450 droplets per second

By using these parameters beads arrived closely packed at the microfluidic junction (Figure 2A) allowing for a high efficiency of encapsulation and a resulting cell capture rate of 67%. The position of the beads inside the droplets is indicated with black arrows, the position of cells inside the droplets with white arrows (Figure 2 B). Approximately 67% of the droplets contained a bead and the droplets were on average 94 µm ± 4.7 % (Figure 2 C).

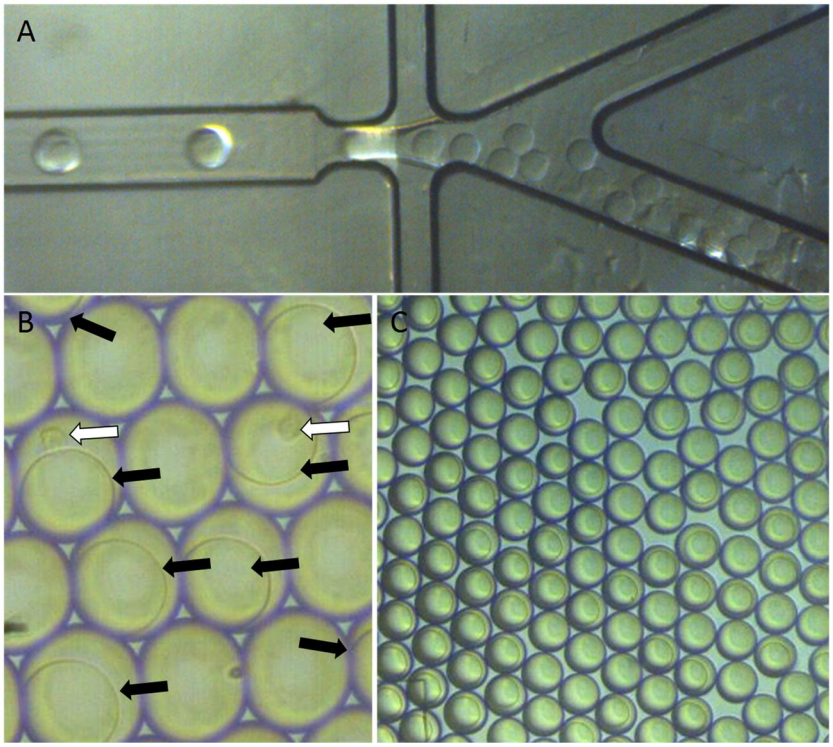

Figure 2

Conclusion

While non-deformable beads for scRNA-seq on Nadia produce high-quality data for a wide range of applications, this approach is less suited for use with limited amounts of cell input material or rare cells due to its low cell capture rate.

In contrast, by using deformable beads, significantly higher capture rates can be achieved as described in Klein *et al.* (2). In this proof of concept study, we have used commercially available deformable beads on the Nadia Innovate. By adjusting parameters such as stirrer speed, pressure tables and flow rates we have achieved similar cell capture rates as previously described. Further studies will be required for optimisation of droplet formation and development of a DropSeq-equivalent protocol on Nadia using deformable beads.

1) Macosko E., *et al.*, "Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets." *Cell* **161**:1202

2) Klein, AM., *et al.* "Droplet Barcoding for Single-Cell Transcriptomics Applied to Embryonic Stem Cells." *Cell* **161**:1187