dolomite bio

High-Throughput Encapsulation of Cells in Individual Micro-Scaffolds with the nadAROSE Kit

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2. SUMMARY

This application note describes the encapsulation of mammalian cells in agarose scaffolds (Figure 1) using the nadAROSE kit, a reagent kit specifically developed to be used on Dolomite Bio's Nadia instrument.

The subsequent recovery of the cell-containing agarose scaffolds, their analysis using flow cytometry and cell release from the agarose scaffolds are also detailed in this document.

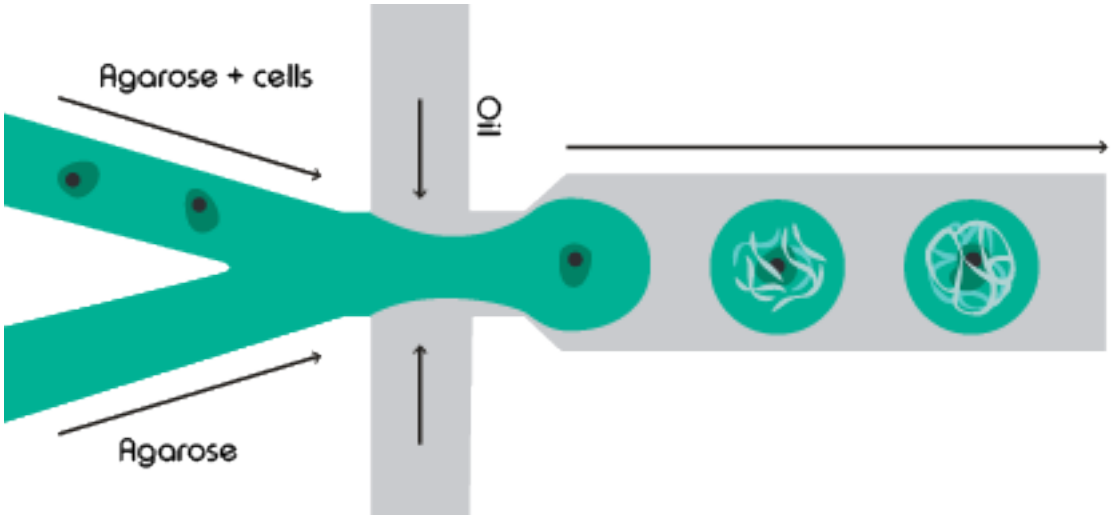

Figure 1: Schematic of cell-containing agarose scaffold generation. *Cells suspended in molten agarose are encapsulated in droplets on the Nadia instrument using flow focussing. The agarose scaffolds are subsequently solidified and removed intact from the oil carrier phase.*

3. INTRODUCTION

Droplets of hydrogel, such as agarose, are picolitre-volume spherical scaffolds which remain stable in an aqueous solution. They represent a huge potential for many single cell applications (1). Agarose encapsulation allows cells to be maintained in individual microenvironments for extended periods of time (2). The properties of agarose scaffolds make them applicable in several scientific fields, such as studying tumour development (3), drug screening (4), bacteriology (5), assessing plant development (6), and many more.

Hydrogel scaffold-based cell delivery systems represent promising vessels for efficient drug delivery. The encapsulation of cells which continuously secrete therapeutics inside hydrogels has clear advantages over microbeads containing only limited concentrations of active drugs. Due to the pico-litre volumes of substrate in which cells can be encapsulated using droplet microfluidics, co-encapsulating living cells in agarose alongside miniscule volumes of active drugs, stressors or growth factors can additionally act as a potent technique for assaying single cell-molecule interactions (7).

Developments in this area of research are enabled by microfluidic devices, such as the Nadia Instrument, which facilitate the co-encapsulation of two distinct cell types in agarose scaffolds, which can in turn be used for high-throughput analysis of cell-cell or cell-pathogen interactions over biologically relevant timeframes. This, coupled with the possibility of subjecting agarose scaffolds to flow cytometry analyses along with their encapsulated contents, means that agarose encapsulation holds great potential in immunology and secretome studies (8).

In addition to its microfluidics capabilities, the Nadia instrument is equipped with an in-built temperature controller that can maintain biologically relevant temperatures anywhere between 1 °C and 40 °C. For agarose, this temperature control means that the hydrogel can be kept in liquid form during droplet formation and solidified afterwards.

This application note describes the encapsulation of mammalian cells in agarose scaffolds using the Nadia Instrument and the nadAROSE kit. The objectives of the experiments were to use the Nadia to encapsulate cells in agarose scaffolds, recover the produced cell-containing scaffolds, analyse them with flow cytometry and show the release of the cells from the scaffolds.

4. MATERIALS AND METHODS

Droplet system. Dolomite Bio's Nadia Instrument is designed to allow high-throughput analysis of single or multiple cells using droplet microfluidics. It produces picolitre-volume droplets using three independent pressure pumps. The Nadia Instrument (Figure 2) has the capability to heat and cool all reagents between 1 °C and 40 °C.

The Nadia Instrument has been designed for ease-of-use, guiding the user through a chosen application with step-by-step instructions that are clear and effortless to follow. Samples are chilled, or warmed, and stirred during the encapsulation process ensuring high quality and homogenous suspensions.

Cell preparation. Mouse 3T3 cells were cultured in DMEM / 10 % FBS / 1X PenStrep until they reached 60-70 % confluency. On the day of experimentation, the culture media was removed, and cells were washed with 10 ml sterile pre-warmed (37 °C) 1X PBS. 1X TrypLE was added and culture flasks were incubated at 37 °C for 3-5 mins to facilitate cell detachment. Double the volume of culture media was added to inactivate TrypLE and the cell suspension was collected in a 15 ml Falcon tube. Cells were centrifuged at 300 x g for 5 mins at room temperature, the supernatant was removed, and the cell pellet was suspended in 1 ml sterile 1X PBS. The centrifugation was repeated for 3 mins, and the cells suspended in 500 µl 1X PBS.

Staining of cells. For agarose encapsulation and cell release experiments, cells were stained using 1 µl 500X Cytopainter (AbCam, #ab176735) per 500 µl of cell suspension. The cells were incubated at 37 °C for 30 mins protected from light. The cells were centrifuged at 300 x g for 5 mins, the supernatant was removed, and the cell pellet was suspended in sterile 1X PBS. For the co-encapsulation experiment, two separate cell populations of 3T3 cells were stained. One population was stained with 20 µM calcein green and the other population was stained using 1 µg/ml Hoechst 33342 stain solution. The cells from each population were passed through a 40 µm cell strainer.

A 10 µl aliquot was loaded into a Neubauer Improved haemocytometer to count the cells and confirm staining had been successful. The cell concentration was adjusted to 5000 cells/µl with sterile 1X PBS. The two populations were then mixed at a 1:1 ratio.

Production of cell-containing agarose scaffolds. Cells were encapsulated in agarose scaffolds using the Nadia instrument and the nadAROSE kit according to the nadAROSE protocol. Cell-containing agarose scaffolds were solidified and released from droplets according to the nadAROSE protocol. Droplets and scaffolds were imaged using the Dolomite Bio high-speed digital microscope or a ZOE Fluorescence BioImager (BioRad).

Figure 2: The Nadia Instrument

Flow cytometry analysis of cell-containing agarose scaffolds. 50 µl of undiluted suspension of scaffolds in PBS and containing 3T3 cells stained with Cytopainter were loaded onto an ImageStream®X Mk II (Luminex) flow cytometer. Images were captured using default settings and analysed using the AI Image Analysis Software.

Dissolution of scaffolds, release of cells and propagation. All recovered scaffolds from an 8-lane Nadia run were pooled into one 1.5 ml microcentrifuge tube (around 100 µl of emulsion are produced per sample when using the nadAROSE kit). In a laminar flow hood, the scaffold suspension was gently mixed up and down and 150 µl was dispensed into 4 wells of a 24-well plate. In the 2 control wells, 100 µl of pre-warmed DMEM (+FBS -P/S) was added. In the 2 test wells, 100 µl of scaffold dissolution mix (0.1 U/µl ß-agarase, 2.5X ß-agarase buffer (NEB, #M0392L) in DMEM (+FBS -P/S)) was added. The plate was placed in a Tissue Culture incubator at 37 °C.

On day 2 of the experiment, the absence of scaffolds in the treated wells was checked using the ZOE microscope. When scaffolds could not be seen, 750 µl of pre-warmed culture media was added to each well to facilitate culture over an extended period. Images were taken from day 0 until day 7.

5. RESULTS

Encapsulation of cells in agarose beads.

Using the nadAROSE kit, 3T3 cells were successfully encapsulated in agarose scaffolds. The droplets exhibited good monodispersity and a median diameter of 75 µm (Figure 3, a).

Following the recovery of scaffolds using a Zerostat anti-static gun, 3T3 cells were observed within agarose scaffolds. The agarose scaffolds presented a median diameter of 75 µm (Figure 3). The size of these agarose scaffolds may make them compatible with cytometric analyses (with large nozzles, i.e., 200 µm).

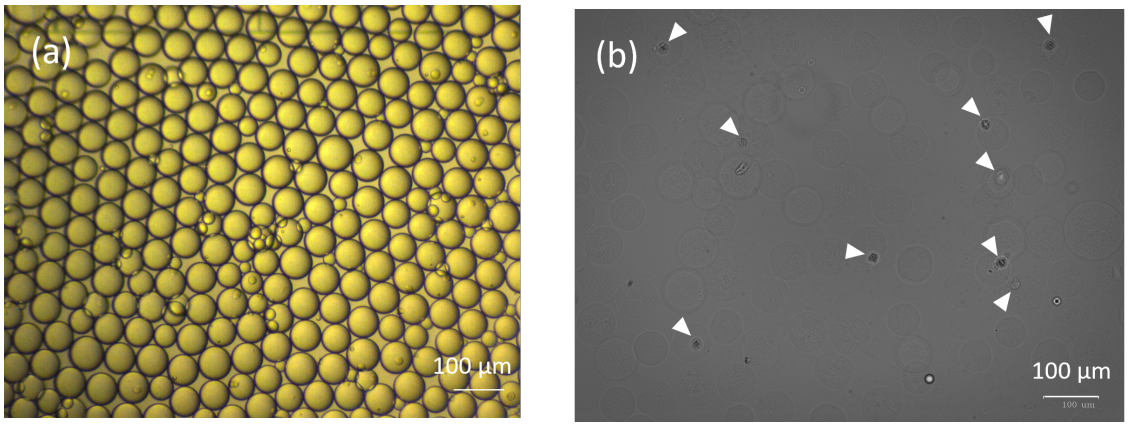

Figure 3 *(a) Emulsion of agarose scaffolds produced with the nadAROSE kit, showing good monodispersity. (b) Agarose scaffolds containing 3T3 cells after recovery.*

Co-encapsulation of cells.

To demonstrate the possibility to co-encapsulate cells, Hoechst and Calcein-stained 3T3 cells were encapsulated together.

Living, differentially-stained, fluorescent cells were observed in the recovered agarose scaffolds (Figure 4).

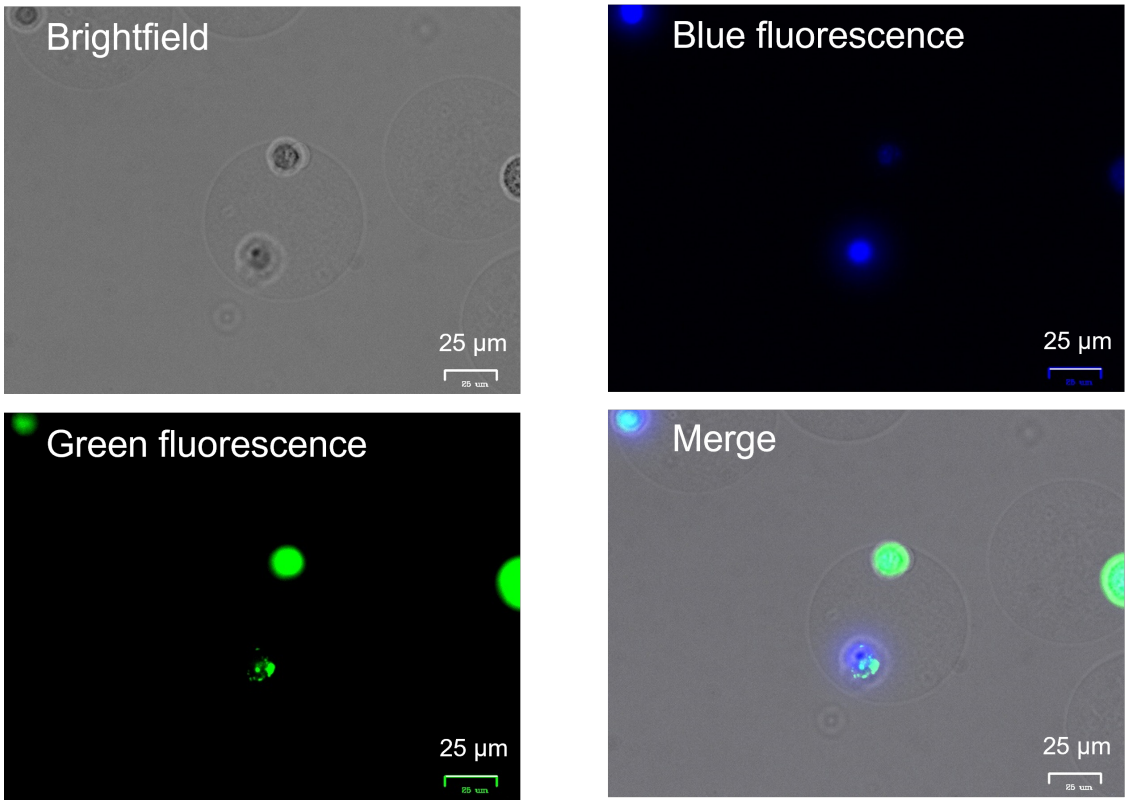

Figure 4: *Hoechst- (in blue) and Calcein-stained (in green) 3T3 cells were encapsulated together. Living, differentially stained, fluorescent cells were observed in the recovered agarose scaffolds.*

Flow cytometry with agarose scaffolds.

Agarose scaffolds containing stained 3T3 cells were analysed using the ImageStream®X Mk II flow cytometer. The instrument was able to detect and distinguish scaffolds containing cells from empty scaffolds based on the fluorescent signal emitted by the cells. The calculated concentration of agarose scaffolds containing cells was 2M/ml. Around 100 µl of scaffolds suspension is produced by each Nadia lane, equating to 200,000 cell-containing scaffolds per Nadia chip or up to 1.6 M scaffolds per Nadia cartridge.

The number of cells introduced into the Nadia chip for producing the scaffolds for flow cytometry was 400,000. This indicates that the recovery rate of cells in agarose scaffolds is approximately 50 %. This number may vary depending on starting cell concentration and how carefully scaffolds are extracted and recovered from the emulsion.

Using a FACS Aria III (BD) machine to sort the agarose scaffolds, we found that a nozzle size of 100 µm is insufficient to allow scaffold sorting. This indicated that bigger size nozzles (200 µm) should be more suitable for sorting scaffolds produced with the nadAROSE kit. Examples of suitable sorters are Influx (BD) and COPAS (Union Biometrica).

Cell release from agarose scaffolds.

Following cell encapsulation, the possibility to dissolve the scaffolds and release the cells was demonstrated by exposing the agarose spheres to ß-agarase.

Scaffolds were visibly dissolved after 2 days and cells maintained viability for 7 days post-cell release. (Figure 5).

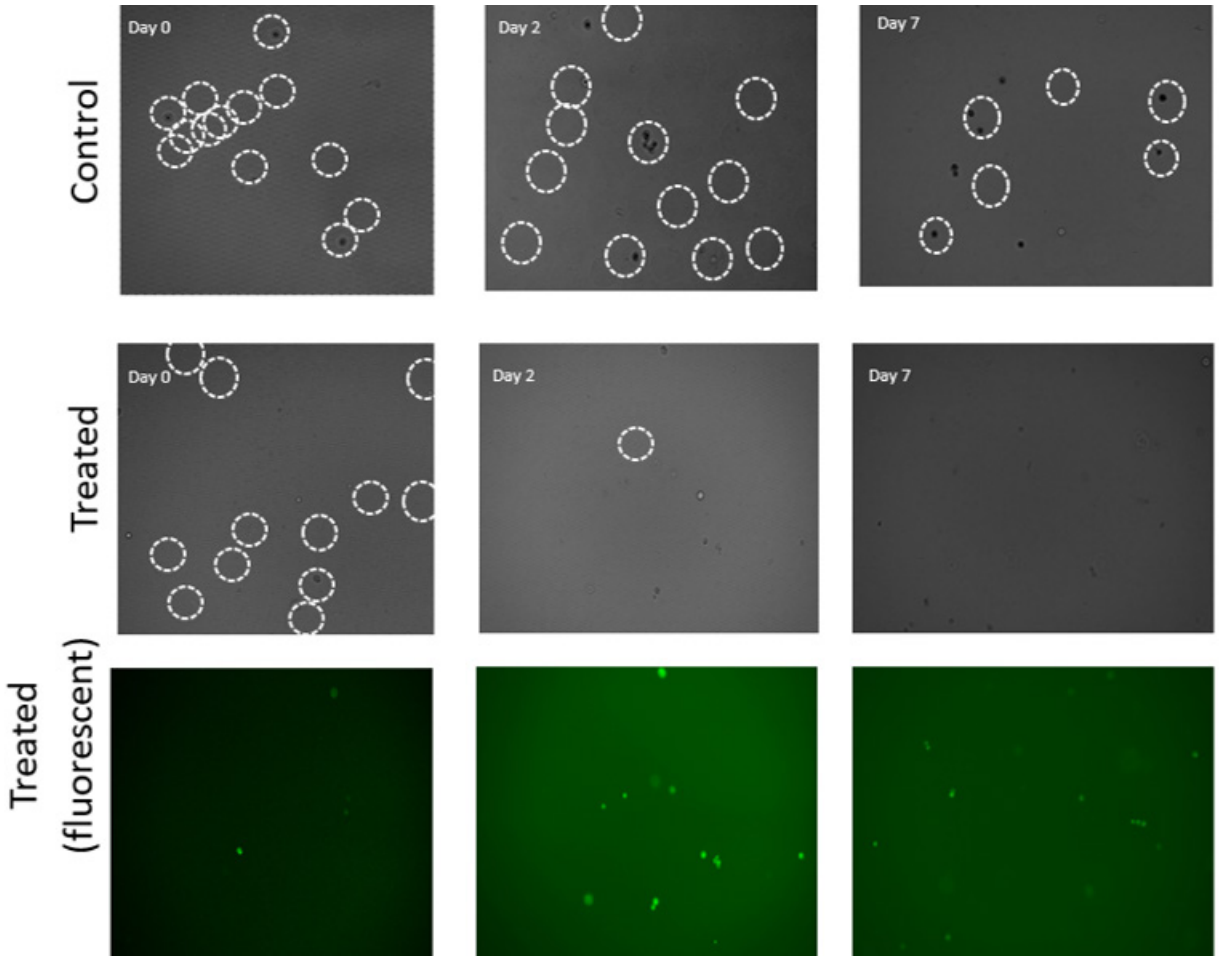

Figure 5: Agarose scaffolds containing cells were successfully dissolved using ß-agarase. *After 2 days, the scaffolds had almost all disappeared (middle). The released cells remained viable (displaying fluorescence) for 7 days after release (bottom).*

6. CONCLUSION

Agarose droplet encapsulation is a useful tool for the individual capturing, immobilisation and imaging of single or multiple cells.

This application note shows that by using the nadAROSE kit with the Nadia instrument, live cells could be readily encapsulated within agarose scaffolds. Examples of downstream processes were demonstrated by showing co-encapsulation of cells, cell release from the scaffolds and flow cytometry analyses.

7. REFERENCES

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8. PRODUCT INFORMATION

Part number	Part Description
3201025	nadAROSE Kit (8 Runs)
3201026	nadAROSE Kit (40 Runs)
3201015	Nadia Cartridge for nadAROSE 8 Runs (8x1)
3201016	Nadia Cartridge for nadAROSE 8 Runs (2x4)
3201017	Nadia Cartridge for nadAROSE 8 Runs (4x2)
3201018	Nadia Cartridge for nadAROSE 8 Runs (2x2 & 1x4)
3201019	Nadia Cartridge for nadAROSE 8 Runs (1x8)
3201020	Nadia Cartridge for nadAROSE 40 Runs (40x1))
3201021	Nadia Cartridge for nadAROSE 40 Runs (10x2 & 5x4)
3201022	Nadia Cartridge for nadAROSE 40 Runs (5x8)

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