dolomite bio

High-Throughput Encapsulation of Cells in 3D Micro-Scaffolds with the nadia3D Kit

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2. SUMMARY

This application note describes the encapsulation of mammalian cells in collagen-based scaffolds, or hydrogel spheres (Figure 1) using the Nadia instrument and the nadia3D kit. The subsequent recovery of the cell-containing collagen-based scaffolds, cell culture and cell release from the scaffolds are also explained in this document.

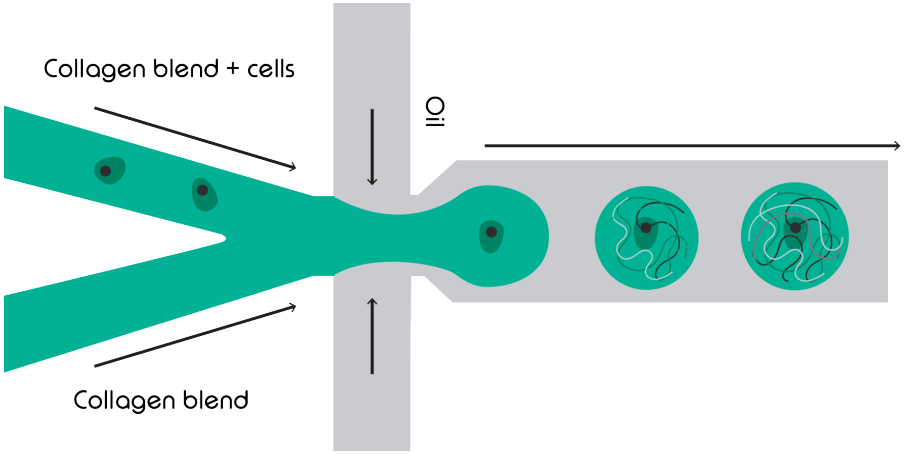

Figure 1. *Schematic of cell-containing collagen-based scaffold generation. Cells suspended in a collagen blend are individually encapsulated in droplets on the Nadia instrument using flow focussing. The collagen-based scaffolds are solidified and removed intact from the oil carrier phase.*

3. INTRODUCTION

A large number of scientific studies that require cell culture rely on 2D culture techniques. These techniques do not successfully mimic in vivo microenvironments of cells. For this reason, many scientists consider 3D culture techniques as a more valuable approach for cell biology studies; whereby cells are encapsulated within hydrogel extracellular matrices where they can grow in a manner more closely representing their native physiological environment (1).

Hydrogel spheres, such as those made of collagen-based gels, are picolitre-volume spherical scaffolds which remain stable in an aqueous solution. They represent a potent solution for many single-cell applications (2). Hydrogels such as collagen also allow the diffusion of nutrients and dissolved gases to circulate and reach encapsulated cells, allowing them to be grown in individual microenvironments for extended periods of time (3). These properties could facilitate cell seeding on to larger scaffolds. High-throughput screening of drugs, which is usually carried out on culture plates on 2D surfaces (4), can also benefit from the use of hydrogel scaffolds. Cells encapsulated in hydrogel spheres alongside miniscule volumes of active drugs, stressors or growth factors can be used to assay single molecule-cell interactions (5). The principle of co-encapsulation within the same scaffold could also be used to study cell-cell or cell-pathogen interactions over biologically relevant timeframes.

Microfluidics, a technology that allows the manipulation of small volumes of fluid in microfabricated hollow channels, can generate such hydrogel scaffolds in a controlled and precise manner (6). Consequently, microfluidic devices, such as the Nadia Instrument, support new developments in the field single cell biology and 3D cell culture. With the built-in temperature controller of the Nadia platform, live cells and hydrogels can be flowed into droplets as a liquid before being solidified into spherical scaffolds. This can be conducted at biologically relevant temperatures anywhere between 1 °C and 40 °C.

This application note describes the encapsulation and cultivation of mammalian cells in collagen-based scaffolds using the Nadia Instrument and the nadia3D kit, demonstrating their potential in the field of single- (or multiple-) cell analysis in hydrogel scaffolds. The objectives of the experiments were firstly to test the encapsulation of cells in collagen-based scaffolds and the recovery of the scaffolds produced from an emulsion, using the reagents provided in the nadia3D kit. Secondly, we demonstrated co-encapsulation of two cell types within a single scaffold highlighting its potential use in cell-cell interaction studies. Thirdly, we showed the ability of mammalian cells to proliferate within the collagen-based gel scaffolds. Finally, we showed the recovery of viable cells from the host scaffolds by dissolving the gel spheres.

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 collagen 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 collagen-based scaffolds. Cells were encapsulated in collagen-based scaffolds using the Nadia instrument and the nadia3D kit according to the nadia3D protocol. Cell-containing collagen-based scaffolds were solidified and released from droplets according to the nadia3D 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*

3D cell culture inside collagen-based scaffolds. In a laminar flow hood, 80 µl of cell-containing scaffold suspension (around 100 µl of emulsion are produced per sample when using the nadia3D kit) was mixed with 920 µl of pre-warmed DMEM (+FBS -P/S). 100 µl of diluted suspension of scaffolds was then dispensed into the wells of an 8-well µ-Slide, or a 24-well culture plate, and topped up with 200 µl or 900 µl of pre-warmed DMEM (+FBS -P/S) respectively. Plates were then placed in a Tissue Culture incubator at 37 °C. Images were taken at regular interval with a ZOE Fluorescence BioImager or a Nikon A1-R Confocal microscope.

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. 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, 400 µl of pre-warmed DMEM (+FBS -P/S) was added. In the 2 test wells, 400 µl of scaffold dissolution mix (0.25 U/µl collagenase (ThermoFisher, #17101015), 1.25 mM CaCl2 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 micro-scaffolds. Using the nadia3D kit, 3T3 cells were successfully encapsulated in collagen-based scaffolds. The droplets exhibited good monodispersity and a median diameter of 90 µm (Figure 3, a). Following the recovery of scaffolds using a Zerostat anti-static gun, 3T3 cells were observed within the collagen-based scaffolds. The scaffolds were of a median diameter of 80 µm (Figure 3, b).

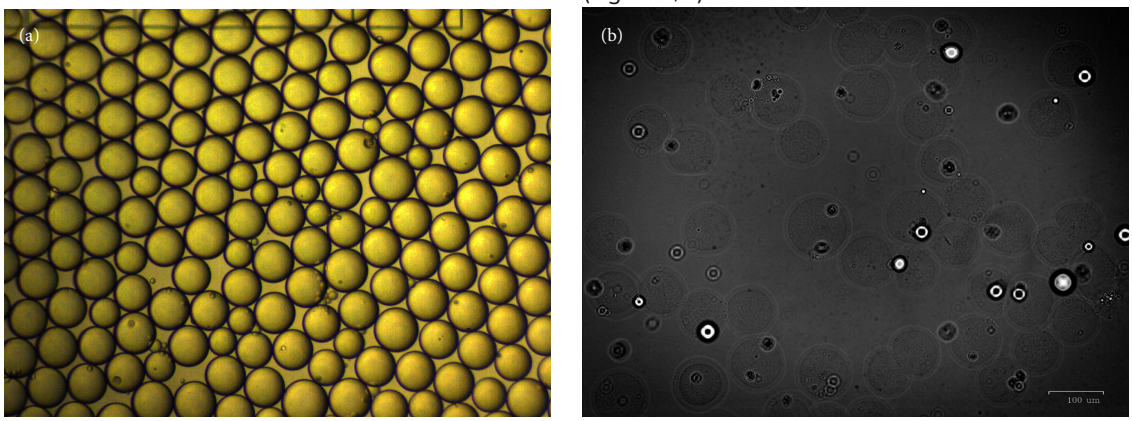

Figure 3 *(a) Emulsion of collagen-based scaffolds produced with the nadia3D kit, showing good monodispersity. (b) Collagen-based scaffolds containing 3T3 cells after recovery.*

Co-encapsulation of cells. To demonstrate co-encapsulation of cells within collagen-based scaffolds, Hoechst and Calcein-stained 3T3 cells were encapsulated together. Both fluorescent cell populations were observed in the recovered scaffolds (Figure 4).

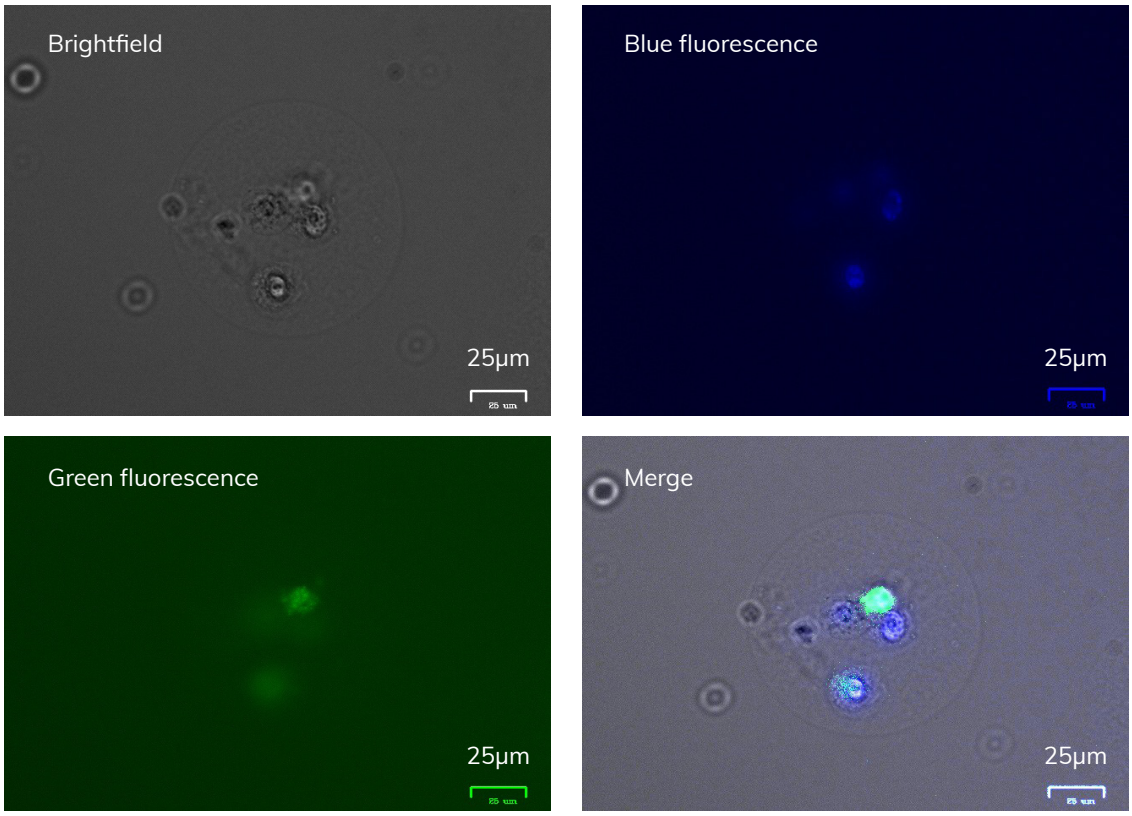

Figure 4: *Hoechst- (blue) and Calcein (green)- stained 3T3 cells were encapsulated together. Live, differentially stained, fluorescent cells were observed in the recovered collagen-based scaffolds.*

3D cell culture in collagen-based scaffolds. Following production of cell-containing collagen-based scaffolds, the capacity for 3D cell culture was demonstrated by observing cell proliferation within the scaffolds over a 7-day period. Towards the end of the 7 days, the cell spheroids became larger than the original scaffold and cells began to escape into the surrounding media (Figure 5).

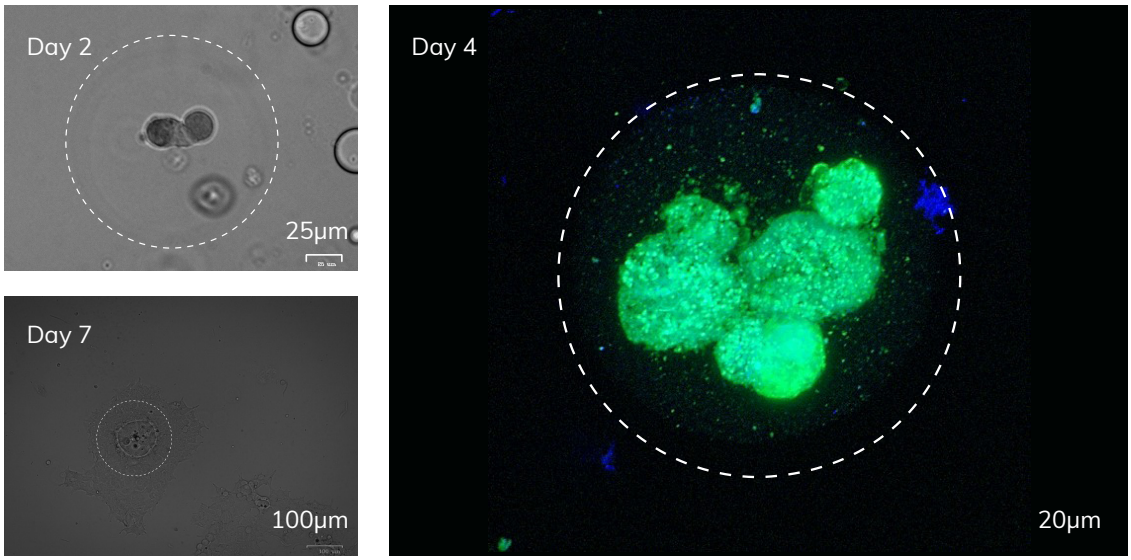

Figure 5: *3T3 cells proliferated inside the collagen-based 3D scaffolds. After 7 days, the cells had begun to escape into the surrounding media. Boundaries of scaffold are highlighted with dashed line.*

Dissociation of scaffolds and cell release. The possibility to dissolve the collagen-based scaffolds was demonstrated by exposing the hydrogel spheres to collagenase. Scaffolds were visibly dissolved after 24h and cells maintained viability for 7 days post-release (Figure 6).

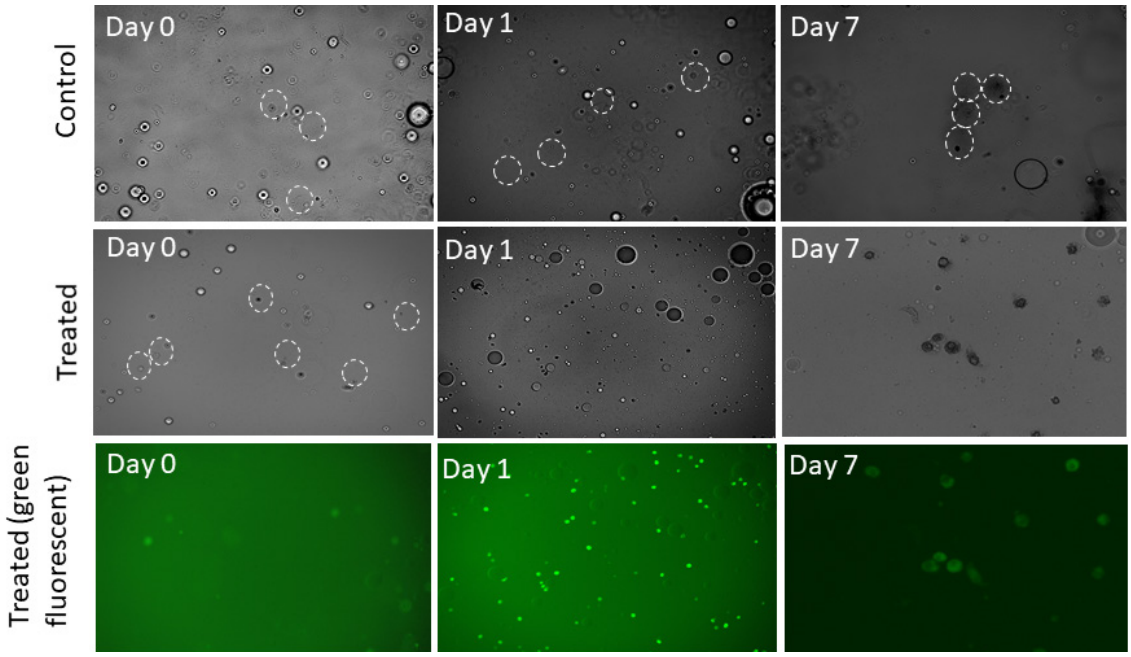

Figure 6: *Release of cells after treatment with collagenase. The treated panel (middle) shows the absence of scaffolds after 24 hrs of treatment while scaffolds persisted within control wells (dashed outline). The released cells remained viable (displaying fluorescence) for 7 days after release (bottom).*

6. CONCLUSION

This application note shows that cells can be readily encapsulated in collagen-based scaffolds using the Nadia Instrument and the nadia3D kit. Cell-containing collagen-based scaffolds can be recovered from oil emulsions and suspended in culture media to allow for cell proliferation. Cells can also be recovered from the scaffolds through enzymatic digestion of hydrogel spheres. The results obtained from these experiments highlight the suitability of the nadia3D kit and Nadia Instrument for long term, controllable 3D cell culture-based downstream applications.

7. REFERENCES

1. Langhans S. Three-Dimensional in Vitro Cell Culture Models in Drug Discovery and Drug Repositioning. Frontiers in Pharmacology. 2018;9.

2. Luo R-C, Chen C-H. Structured Microgels through Microfluidic Assembly and Their Biomedical Applications. Soft. 2012;01(01):1–23.

3. Joseph JS, Malindisa ST, Ntwasa M. Two-Dimensional (2D) and Three-Dimensional (3D) Cell Culturing in Drug Discovery. In: Cell Culture [Internet]. 2018 [cited 2019 Sep 19]. Available from: https://www.intechopen.com/books/cellculture/two-dimensional-2d-and-three-dimensional-3d-cell-culturing-in-drugdiscovery

4. Zhu Z, Yang CJ. Hydrogel Droplet Microfluidics for High-Throughput Single Molecule/Cell Analysis. Acc Chem Res. 2017;50(1):22–31.

5. Whitesides G. The origins and the future of microfluidics. Nature. 2006;442(7101):368-373.

6. Pampaloni F, Reynaud E, Stelzer E. The third dimension bridges the gap between cell culture and live tissue. Nature Reviews Molecular Cell Biology. 2007;8(10):839-845.

8. PRODUCT INFORMATION

Part number	Part Description
3201023	nadia3D Kit (8 Runs)
3201024	nadia3D Kit (40 Runs)
3201007	Nadia Cartridge for nadia3D 8 Runs (8x1)
3201008	Nadia Cartridge for nadia3D 8 Runs (2x4)
3201009	Nadia Cartridge for nadia3D 8 Runs (4x2)
3201010	Nadia Cartridge for nadia3D 8 Runs (2x2 & 1x4)
3201011	Nadia Cartridge for nadia3D 8 Runs (1x8)
3201012	Nadia Cartridge for nadia3D 40 Runs (40x1)
3201013	Nadia Cartridge for nadia3D 40 Runs (10x2 & 5x4)
3201014	Nadia Cartridge for nadia3D 40 Runs (5x8)

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