Holotomography-monitored phagocytosis assay of M1 macrophages

PromoCell®

Application note

Background

Macrophages are tissue-resident professional phagocytes and antigen-presenting cells (APC) that are derived from circulating peripheral blood monocytes. They perform important active and regulatory functions in both adaptive and innate immunity, e.g. by clearing bacterial infections from open wounds.

Macrophages are able to recognize bacteria by activating special cell surface receptors bind to various components of bacterial cell walls. A bacterium is engulfed by the macrophage's cell membrane and the resulting vesicle constricted to form a so-called phagosome that is moved into the macrophage's cytoplasm. There it undergoes a maturation process and enters the phagolysosomal pathway, eventually leading to its degradation and complete clearance. The phagosome fuses with a lysosome, which is an acidic intracellular vesicle containing large amounts of hydrolytic enzymes, form a cytoplasmic body called a phagolysosome. It therefore also has an acidic pH, thus providing an optimal environment for enzymatic degradation of bacterial pathogens.

Phagocytosis Assay Kit (*E. coli*)

To test the viability of macrophages in vitro, a phagocytosis assay is an excellent way to determine whether they are working properly. Phagocytosis assays often use E. coli and other bacterial strains as the pathogens. PromoCell's Phagocytosis Assay Kit (product discontinued) uses heat-killed, red or green fluorescently labeled *E. coli* particles for fast, accurate detection and quantification of in vitro phagocytosis. The kit provides a robust screening system for all activators and/or inhibitors of phagocytosis and toll-like receptor (TLR) ligands. Analysis can be performed using various microscopy techniques, spectrophotometry, or flow cytometry.

Nanolive's Cell Explorer for live cell holotomography

Nanolive imaging is a versatile observational tool. It is a technique for measuring a three-dimensional refractive index tomogram of a microscopic specimen that eliminates the need to use fluorescent labels or modify the physiological environment of the cells *in vitro*. Cellular processes can be directly monitored and analyzed. Rotational scanning allows 3D reconstructions while providing noise robustness and a resolution far greater than is possible with standard light microscopy.

The 3D Cell Explorer from Nanolive uses a globally unique technology that has been patented in the U.S. (no. 8,937,722) and European Union (publication no. WO/2011/121523).

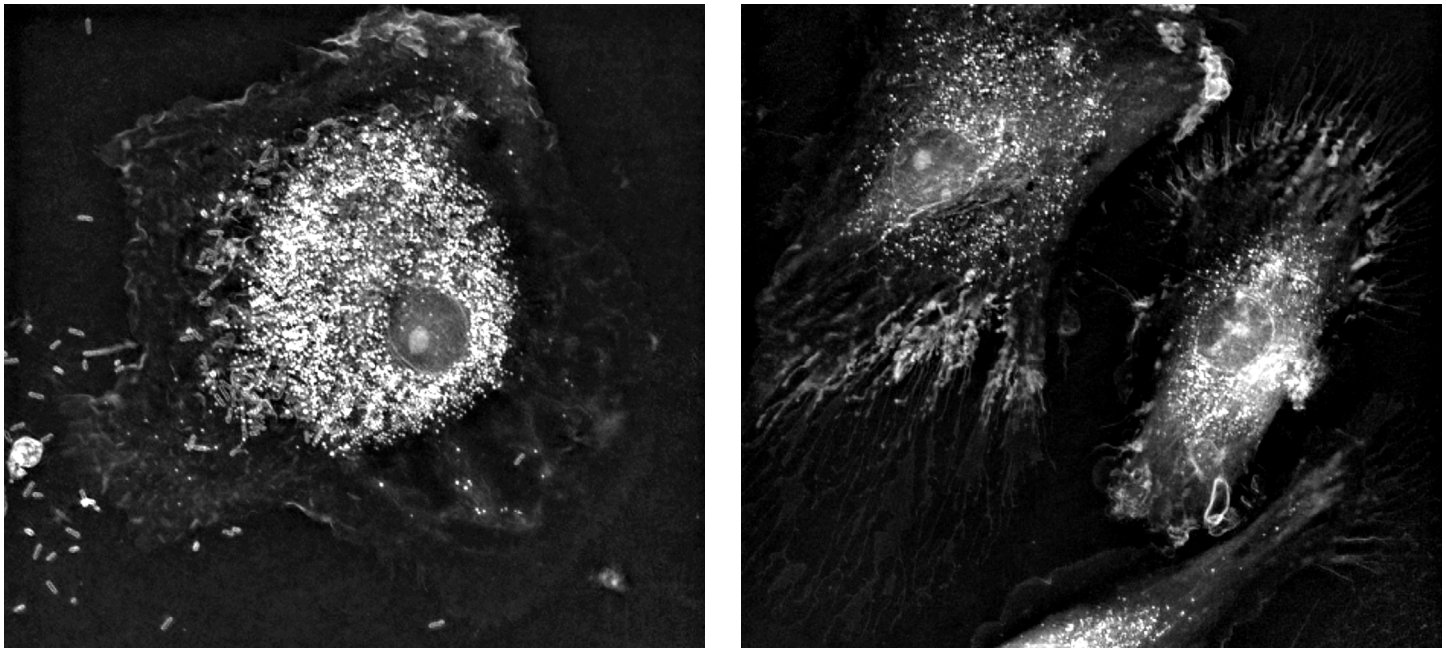

Fig. 1: Cryopreserved human M1 macrophages (GM-CSF). *Macrophages were plated with fibronectin and monitored in the Cell Explorer workstation (5% CO_2). The experiments were performed during 24 hours, with a frame rate of 6Hz and a field of view of 85 × 85 × 30 µm. Watch the live footage here.* ***A:*** *M1 macrophage phagocytoses E.coli (inside and outside of the cell).* ***B:*** *Macrophage movement cultured in M1-Macrophage Generation Medium XF on glass-bottom cell culture vessels.*

Phagocytosis assay protocol

I. Phagocytosis Assay Kit (*E. coli*) protocol

Materials

- Proliferating culture of human macrophages in good condition (C-12914 or C-12916)
- M1- or M2-Macrophage Generation Medium XF (C-28055 or C-28056)
- Phagocytosis Assay Kit (*E. coli*)
- 3D Cell Explorer workstation and its controlling software STEVE (https://nanolive.ch/cx/)
- Glass-bottom dishes or cell culture vessels compatible with the 3D Cell Explorer
- Centrifuge

Use aseptic techniques and a laminar flow bench.

II. Storage and reagent preparation

- Store the entire kit at 4°C, protected from light.
- Phagocytosis assay buffer: Upon arrival, combine one entire vial of buffer additive with one bottle of phagocytosis assay buffer and mix well.
- Green *E. coli:* Before use, acclimate the suspension to room temperature and vortex gently for 5 seconds.
- Quenching solution: Dilute the content of the vial into 4.5 ml of 1x phagocytosis assay buffer

III. Assay

1

Preparation of control and experimental wells

Subculture cells capable of phagocytosis in appropriate medium. Please read more in our Cryopreserved macrophage application note.

2

Day -1

Determine viability of cells (e.g. using the colorimetric WST-8, the fluorometric resazurin or the bioluminescent ATP-based assay) and resuspend cells to a concentration of 1 – 5 × 10^6 viable cells/ml. Aliquot 100 µl of the cell culture per well omitting the negative control wells and incubate the plate overnight at 37°C, 5% CO_2.

3

Day 0

Change the media and proceed to the phagocytosis effector assay. Your experiment should always include parallel negative, positive and experimental wells.

4

Additional phagocytosis effector assay

Add 100 µl of cell culture media containing your effector of interest at the desired concentration (e.g. 20 µM Cytochalasin D) to each of the experimental wells. Aliquot 100 µl of media to each of the positive and 200 µl media in each of the negative control wells respectively. Incubate for 1 hour at 37°C, 5% CO_2.

5

Phagocytosis of *E. coli*

Add 5 µl of E. coli slurry to all the wells. Immediately transfer the plate back to the incubator for 2 – 3 hours. The incubation time may be adjusted according to your protocol.

6

E. coli Standard curve

Add 0, 1, 2, 3 and 4 µl of green *E. coli* slurry to the cell culture vessel. Adjust the volume to 100 µl with phagocytosis assay buffer. Mix well. Immediately measure and plot the standard curve.

7

Detection

Cell activity can be analyzed by holotomography, FACS or other microscopic techniques.

IV. Live cell analysis with the 3D cell explorer

1

Preparation

Allow proliferating culture of human macrophages to grow on glass-bottom dishes compatible with the 3D Cell Explorer.

2

Live observation of phagocytosis of M1 macrophages

Prepare the 3D Cell Explorer workstation and its controling software STEVE. Image frame rate 6Hz, experimental setup, STEVE, FOV of 85 x 85 × 30 µm, air objective, 60x.

3

Possible parameters for analyzing and determining the viability of macrophages

Change the media and proceed to the phagocytosis effector assay. Your experiment should always include parallel negative, positive and experimental wells.

- Percentage of macrophages containing bacteria
- Average number of intracellular bacteria per macrophage
- Percentage of macrophages with attached bacteria
- Average number of attached bacteria per macrophage

Products

Media	Size	Catalog number
Human M1 Macrophages (GM-CSF), monocyte-derived, single donor	1.5 Mio/5 Mio	C-12914/C-12916
Human M2 Macrophages (M-CSF), monocyte-derived, single donor	1.5 Mio/5 Mio	C-12915/C-12917
Fibronectin Solution, human (1 mg/ml)	5 ml	C-43060
M1-Macrophage Generation Medium XF (incl. GM-CSF)	250 ml	C-28055
M2-Macrophage Generation Medium XF (incl. M-CSF)	250 ml	C-28056
3D Cell Explorer	Holotomographic microscope	nanolive.ch/cx

References

1. Ovchinnikov, D.A., et al., Macrophages in the embryo and beyond: much more than just giant phagocytes. Genesis, 2008 46(9): p. 447–62. doi:10.1002/dvg.20417.
2. Kloc, Macrophages Origin, Functions and Biointervention. Book, Springer, 2017, ISBN 978–3–319–54090–0

This is a collaboration of:

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PromoCell®

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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