Flow Cytometric Quantification to Assess Dorsal Endocytosis

APPLICATION NOTE

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IN THIS PAPER YOU WILL LEARN

- How to setup a phagocytosis assay
- How to measure cellular phagocytosis by flow cytometry
- How to set up controls and properly gate a phagocytosis assay using flow cytometry

Background

The accumulation of collagen during fibrosis is caused by excessive production and reduced degradation. To test the ability of fibroblasts to uptake and digest collagen, we used a flow cytometry application that examines the uptake of beads coated with collagen type I under different fibrotic cell culture models.

Principal of the Technique

Collagen coated bead phagocytosis assay to assess collagen phagocytosis

Here, as a proof of principal we used rat embryonic fibroblasts (REF52) to examine phagocytosis of collagen type I coated fluorescent microspheres. Red fluorescent latex beads (maximally excited at 580 nm but excited using the 488 nm laser on the standard CytoFLEX) coated with collagen were incubated with REF52 cells for 10, 30 minutes and 1 hour to assess bead uptake by cells as a measure of collagen phagocytosis, the bead signal was detected in the 660/20 filter.

The use of a flow cytometry approach to quantify cellular phagocytosis is beneficial as it allows for a quick assessment of cellular uptake in a large number of cells and can be applied to other cell types such as macrophages or neutrophils (‘professional’ phagocytic cells).

Protocol

Cell Line and Cell culture

REF52-WT cells (rat embryonic fibroblasts, W. Topp, Cold Spring Harbor Laboratory) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10 % fetal bovine serum and 1 % Penicillin/Streptomycin.
In preparation for the experiment REF52 cells were seeded at a density of $1.5x10^5$ cells per 60 mm dish and cultured overnight.

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Bead Coating with Collagen type I

Stock 1 µm red FluorSphere beads 2% w/v (bead volumes must be individually optimized) were washed three times in PBS supplemented with 0.02 % TX-100. Following the washing step beads were re-suspend in Collagen type I solution (1 mg/mL) and incubated at 37°C for 1.5 h, with frequent agitation. Collagen coated beads were then washed, resuspended in DMEM (serum-free), sonicated and used immediately.

Flow Cytometry for Bead Endocytosis

Beads were added to the cells drop wise and incubated for 10, 30 min and 1 h. Following incubation with collagen coated beads cells were washed twice with PBS, trypsinized with 0.25 % trypsin-EDTA, and collected by centrifugation. Cell pellet was then washed with PBS, resuspend in ice-cold PBS containing 2 % FBS. Samples were then strained into a 5 mL polystyrene round bottom tube with a cell strainer cap (Falcon), samples were then analyzed on CytoFlex (Beckman-Coulter), red laser, 660/20 filter configuration.

Materials & Methods

- DMEM (Gibco-BRL) (supplemented with 10 % FBS + 1 % Penacillin/Streptomycin)
- 0.25 % Trypsin-EDTA (Gibco-BRL)
- 1xPBS (Gibco-BRL)
- 1 µm red FluoroSphere beads, 580/605 (excitation/emission) (Molecular Probes)
- Collagen type I (Sigma)
- 5 mL polystyrene round bottom tube with cells strainer cap (Falcon)

Tissue Culture source:

Sample Type (include cell line information if available)	Species	Age of specimen (if available- or time since prep)	Prep Method
REF52-WT	Rat	Unknown, immortalized cell line	As described in material and methods

Result

The use of a flow cytometry approach for the quantification of collagen I coated bead uptake is a rapid and useful approach to examine collagen clearance in cells undergoing fibrotic differentiation. In combination with other techniques it will provide important information about the mechanism and kinetics of this integral component of wound healing and repair process.

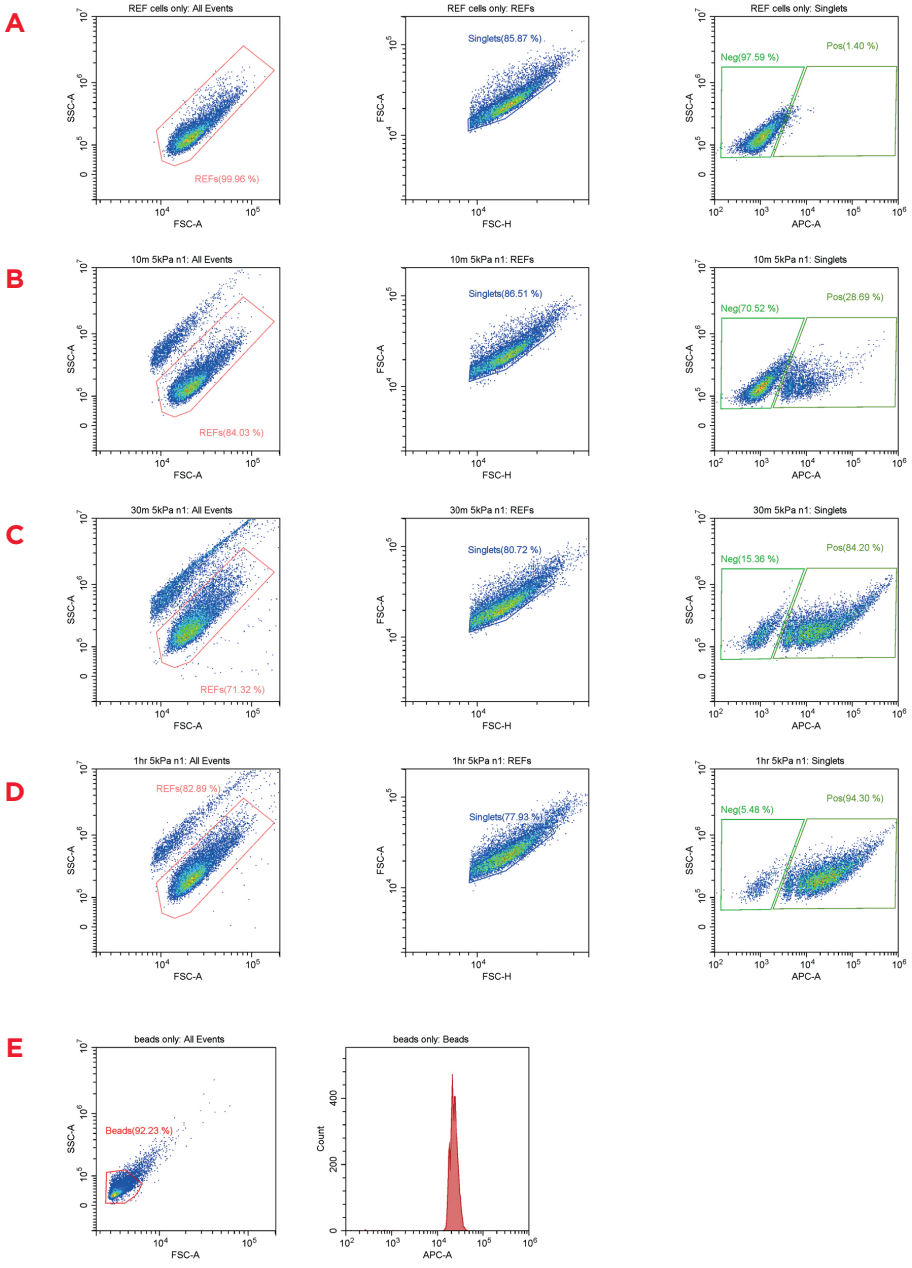

Figure 1: Time course of fluorescent bead phagocytosis by REF52 cells, (beads were coated with Collagen I).

The uptake of fluorescent beads by REF52 cells was identified through hierarchical gating by first selecting the total cell population (SSC-A vs. FSC-A), then by gating on single cell events (FSC-A vs. FSC-H), finally using the (SSC-A vs. APC-C) parameter to separate negative vs. positive population of cells for bead uptake.

A- REF52 cells only, time course:

B- 10min.

C- 30min.

D- 1h.

E- Beads only control.