Foam cell formation

(In vitro）

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Date:12/10/2013

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Macrophage model establishment

Cell origin : primary macrophage(such as peritoneal macrophage, bone marrow-derived macrophage); macrophage cell lines(such as THP-1)

Medium: RPMI 1640 with 10% FBS and antibiotic

Condition：37℃， 5%CO_2

Cell density: 10^6/ml for primary cells, and 0.2～1.0×10^6/ml for macrophage cell lines

mouse peritoneal macrophage

1. The mice are sacrificed by cervical dislocaton;
2. Sterile ice-cold PBS is injected into the cavity of each mouse by peritoneal lavage;
3. The fluid is carefully collected and centrifuged at 1000rmp for 6min;
4. The supernatant is then withdrawn, and the cell pellet is resuspended in RPMI 1640 medium (containing 100 IU/ml of penicillin, 100 ug/ml of streptomycin and 100 ug/ml of L-glutamine);
5. Then plated in 6-well plates at 1.5 $\times 10^6$ cells per well;
6. Incubated in 37 ℃, 5%CO_2 for 2-3h to allow adherence;
7. Non-adherent cells are rinsed away with pre-warmed RPMI 1640 and 2ml of complete RPMI 1640 medium(10%FBS) is added;
8. Medium with all additions are replaced daily and macrophage are used within 5days from harvesting.

Flow cytometry

Introduction: a laser-based, biophysical technology employed in cell counting, cell sorting, biomarker detection and protein engineering, by suspending cells in a stream of fluid and passing them by an electronic detection apparatus.

Purpose: evaluate the purity of macrophages
Cell marker: murine(CD11b,F4/80)
human(CD14, CD68)

Foam cell model establishment

- Modified LDLs: acetylated LDL, oxidized LDL
- Varying levels of oxidation of LDL: reflected by different levels of TBars
- An initial dose titration to determine the optimal dose of modified LDL and the optimal incubation time to produce foam cell
- Positive control: incubated with another form modified LDL to confirm that the macrophage cells are capable of forming foam cell
- Negative control: incubated with LDL for foam cell formation

procedure

✓Plated macrophages are exposed to 50ug/ml oxidized LDLs in 2ml RPMI 1640 medium with 3% FBS and antibiotic;

✓Incubate for 48h at 37 ℃, 5%$CO_{2;}$

✓After 48h, detect the foam cell formation

How to detect the foam cell formation?

✓By light microscopy after staining with Oil Red O

✓By fluorescence microscopy after staining with Nile Red

✓High power phase contrast microscopy (sufficient numbers of lipids droplets are present)

Oil Red O stain

Staining solution:

Oil Red O: 12ml Oil Red O stock solution plus 8ml of distilled water(filtered to remove any particulates)

hematoxylin

Principle:

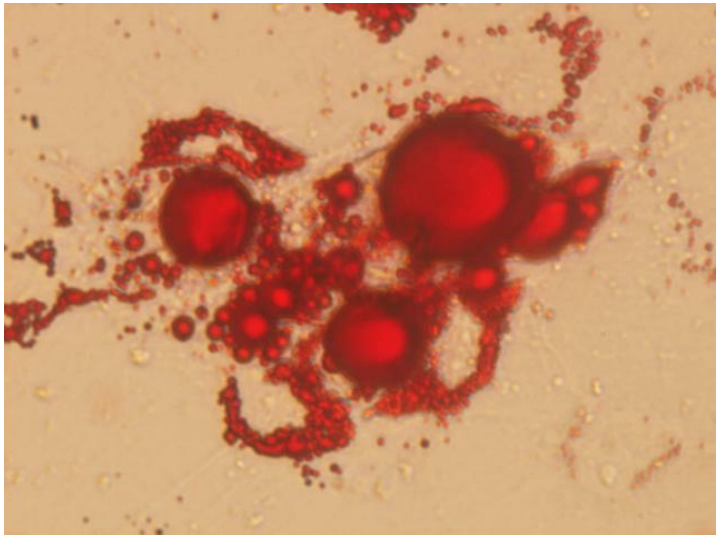
Oil Red O: The dye exhibits a greater solubility in lipoid substances than that in original solvents. So during staining, the dye will migrate into lipids from organic solvents resulting in lipid staining.

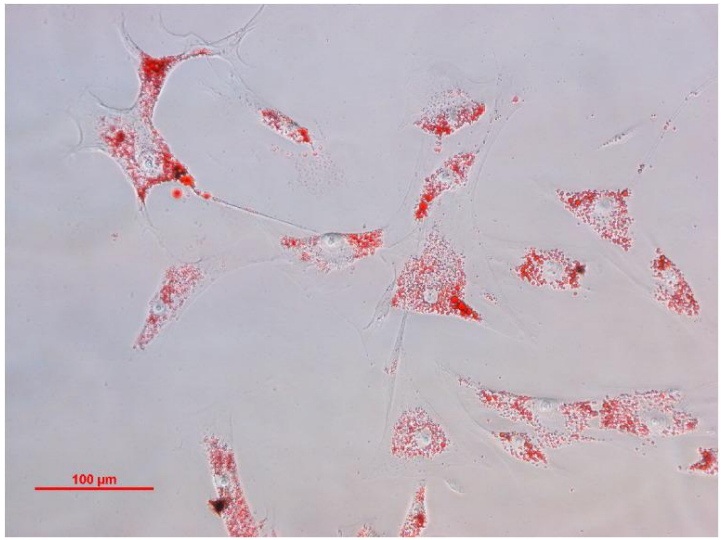
hematoxylin: positively charged metal-hematein complexes bind in an acid milieu to the negatively charged phosphate remains of the cell nucleus-DNA.

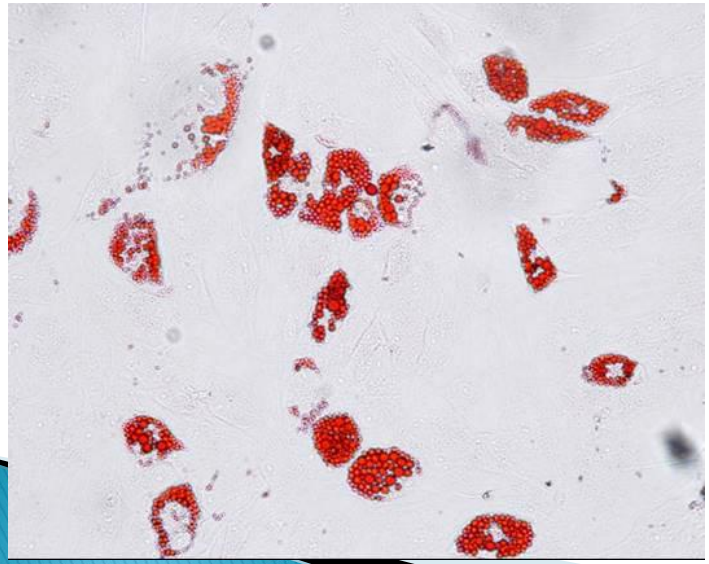
procedure

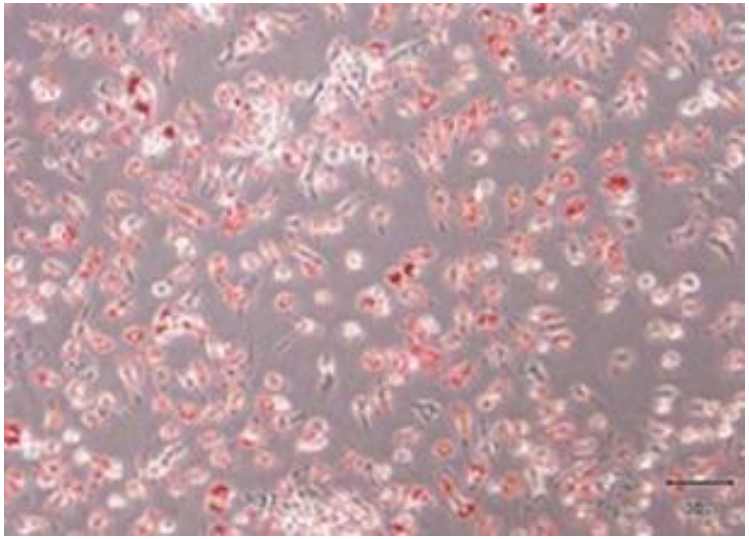
1. rinsed twice with PBS PH7.4;
2. Fixed with 3% paraformaldehyde in PBS for 5min at room temperature;
3. Rinsed once with PBS and the cells covered briefly with Oil Red O/60% isopropanol;
4. Rinsed quickly twice with distilled water to remove excess Oil Red O;
5. Counterstained with hematoxylin for 4min and rinsed with distilled water
6. Cover slips are picked up with tweezers, dipped into distilled water, excess water drawn off with the edge of a Kim Wipe;
7. Then placed cell side down on a drop of mounting medium (90%glycerol, 10%PBS) on a glass microscope slide ;
8. The edges of the cover slip can be sealed with melted paraffin wax.

Foam cell under light microscopy (Oil Red O)









Quantitative assay

Analytical method

liquid chromatography coupled with flame ionization or mass spectrometry detection, such as HPLC-MS

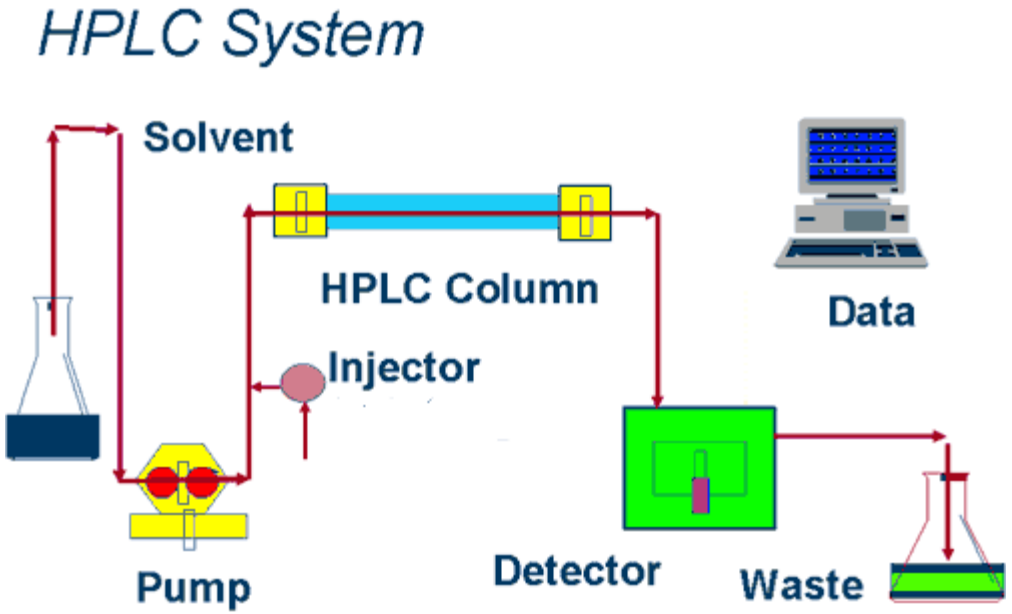
Enzymatic assay

colorimetric or fluorometric

HPLC-MS

Characteristic:

- accurate but time consuming
- process sample one at a time
- chemical saponification of cholesterol esters



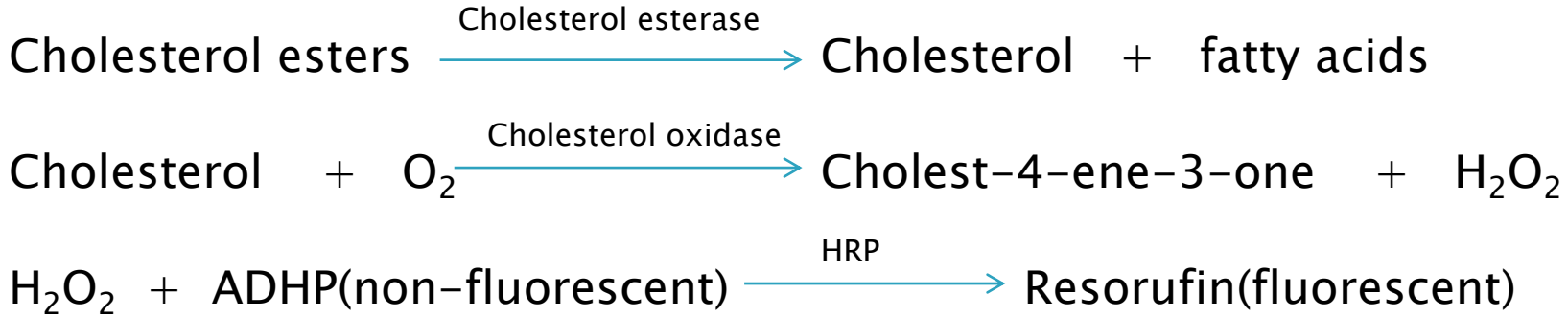
Procedure(total cholesterol)

1. Washed three times with cold PBS and the cells are scraped from each well of a 6-well plate;
2. Resuspended in 1ml cold saline and repeat freeze thawing three times;
3. Sonicated in ice bath for 5min and the cell lysate is then subpackaged (200ul every part);
4. 300ul of freshly prepared 15% alcohol-KOH is added to 200ul of cell lysate;
5. 2 hours later, add 500ul hexane:isopropanol (4:1)solution ;
6. After 15min vortex-mixing, centrifuged at 4000 rpm for 5min and the upper organic phase is collected;
7. Residual free cholesterol in the lower aqueous phase is extracted twice as described above;
8. The total clarified supernatants are collected and evaporated to dryness by vacuum centrifugation;
9. The dry sample is dissolved with a stigmasterol solution(10ug/ml, as an internal standard) and analysed using the LC-MS method.

Enzymatic Cholesterol Assay

Characteristic: simple, sensitive , relatively fast assay, and process many samples at a time

Principle:



Free cholesterol measured by omitting the first esterase step

THANKS FOR YOUR ATTENTION!