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Working

UC Davis - Macrovascular Permeability and Lipoprotein Flux

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ABSTRACT

Summary:

One of the three indices of arterial function that are compromised to a varying degree in individuals with cardiovascular disease is vascular permeability. This assay measures vascular permeability (as flux of labeled large molecular weight molecules: i.e. albumin or dextran) and lipid permeability (as flux of labeled lipid) in coronary or carotid arteries.

Modified from: Walsh et. al. Arterioscler Thromb Vasc Biol. 1999 Apr;19(4):840-6.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=107&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Krebs-Henseleit Solution	See Below	
FITC- Dextran	FD4, FD40S, or FD70	Sigma Aldrich
TRITC- Dextran	T1037 or T1162	Sigma Aldrich
FITC- Albumin	A9771	Sigma Aldrich
TRITC- Albumin	A2289	Sigma Aldrich
Alexa-546 label	10237	Sigma Aldrich
DiL labeled Lipid	See protocol	
pentobarbital		Cardinal Health
DMEM	11885	Invitrogen - Thermo Fisher

MATERIALS TEXT

Reagent Preparation:

Reagent 1: Krebs-Henseleit Solution
116 mM NaCl, 5 mM KCl, 2.4 mM $CaCl_2*H_2O$, 1.2 mM $MgCl_2$, 1.2 mM NH_2PO_4, and 11mM glucose

Note:
Sigma-Aldrich, RRID:SCR_008988

SAFETY WARNINGS

WARNING:

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.

1 Mice are anesthetized with an intraperitoneal injection with 50 mg pentobarbital/kg weight.

2 Blood was collected from each animal through the right atrium using a 22-gauge needle and a heparinized syringe. Blood was transferred to sterile Vacutainers and centrifuged at 2800 rpm for 10 min. Plasma samples were separated from blood cells and kept at 4°C.

3 Vasculature was flushed with DMEM by infusion into the left ventricle of heart.

4 Either common carotid arteries or aorta are dissected and canulated.

a. An anterior midline skin incision was made from the mandible to the sternum and superficial neck muscles were retracted and the carotid arteries were carefully dissected free from surrounding tissue

b. An incision was made in the proximal artery and a cannula (polyethylene-50 tubing) was inserted and tied into place with 4-0 silk

c. A second incision was made in the distal portion of the artery, just proximal to the bifurcation of the common carotid artery, and another cannula was inserted and tied into place

5 Artery in perfused with Kreb's-Henseleit solution plus 1% bovine serum albumin gassed with 95% compressed air and 5% CO_2 until the start of the experiment.
Note: During our experiments, the artery is maintained at a constant length from the moment it is cannulated until the time it (via the cannulae) is secured into place in the perfusion chamber.

6 Cannulated artery was placed in a clear fluid-filled superfusate chamber and mounted on a Nikon MM-11 upright microscope stage for viewing.

7 Perfusate flowed through the artery at a physiological flow rate (1.5-2ml/min) and hydrostatic pressure (90 cm H_2O). All perfusates are maintained at 37°C and pH 7.3-7.4. Perfusate included:

a. Clear, non-fluorescent solution of Kreb's-Henseleit solution with 1% BSA

b. FITC- or TRITCI- labeled Dextran or Albumin at 40-70 µg/mL in Kreb's-Henseleit solution with 1% BSA

c. DiL (see previous protocol) or Alex-546 (according to manufactures instructions) labeled lipid particles at 50 µg/mL in Kreb's-Henseleit solution with 1% BSA.

8 The artery in the superfusate chamber is brought into focus using a Nikon Plan X4 objective (NA 0.1). Fluorophore is excited and emission (see chart below) measured by a Nikon P1 photometer and input to a chart recorder and computer and visualized by Hamamatsu CCD television and camera.

Label	Excitation	Emission
DiL	549	565
FITC	495	521
TRITC	494	518
Alexa-546	554	570

9 The artery is perfused with the nonfluorescent solution to determine a baseline level of fluorescence intensity (If).

10 The artery is then perfused with the buffer solution containing the fluorescently labeled compound (FLC) for 10 min, immediately followed by wash out (10 min) of the fluorescent solution with the clear non-fluorescent solution.

a. Permeability: FITC-or TRITC- labeled albumin or dextran

b. Lipoprotein Flux: Dil or Alex546 labeled lipoproteins

11 If_0 represents the initial fluorescence intensity of the solution filling the artery lumen and IfA represents change in fluorescent intensity between baseline (If) and after washout (accumulation) all measures in milliVolts (mV).

12 **Calculation of flux (ng/min/cm²)**

$Artery_V = \pi r^2 l$ $Artery_{SA} = 2\pi r l$

$Artery_V$ * [FLC in ng/mL] = ng in vessel at If_0

ng in vessel at If_0 / If_0 (mV) = ng/mV

ng/mV * If_A (mV) = ng compound at If_A

ng of compound at If_A /time(min)/ $Artery_{SA}$ = ng/min/cm²