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Preparation of human Red Blood Cells for Scanning Electron Microscopy imaging

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ABSTRACT

SEM imaging can be used to quantify RBC shape. RBCs are sampled from fresh blood samples or from packed RBC units during storage. RBCs are resuspended into PBS to make up 500 μ L of solution at 5% haematocrit. RBCs are then fixed by progressively adding a concentrated 2% glutaraldehyde solution in the cell suspension. The cells are incubated for 30 min at RT and in the dark, before being centrifuged and washed in PBS. This fixation protocol was established in order to limit RBC shape changes due to the presence of glutaraldehyde.

After fixation, a RBC suspension at 2.5% haematocrit is adhered to coverslips coated with poly-D-lysine. A second fixation step is realised on the adhered RBCs by incubating the coverslips in osmium tetroxide in cacodylate buffer (1%) for one hour. The coverslips are then dried by incubating them in an ascending series of ethanol, then by incubating them with hexamethyldisilazane (HMDS) for 30 min. The HMDS incubation step is repeated twice before the coverslips are dried in open air. Finally, the coverslips are gold coated and imaged with a Zeiss Sigma FESEM.

MATERIALS

MATERIALS

- ✂ Glutaraldehyde EM Grade 25% **Sigma Aldrich Catalog #G5882-50ML**
- ✂ Ethanol 100% **Contributed by users**
- ✂ MilliQ Water **Contributed by users**
- ✂ PBS **Lonza Catalog #17-517Q**
- ✂ Poly-D-Lysine **Thermo Fisher Scientific Catalog #A3890401**
- ✂ Sodium cacodylate trihydrate **ProSciTech**
- ✂ Osmium Tetroxide **ProSciTech**
- ✂ Hexamethyldisilazane **ProSciTech**

Fresh or stored red blood cells (RBC)

Protocol status: Working

SAFETY WARNINGS

Created: Mar 06, 2019

All fixation steps should be conducted under a fume hood. Check SDS before starting work.

Last Modified: Apr 20, 2019

PROTOCOL integer ID:
21129

BEFORE START INSTRUCTIONS

Keywords: Red blood cell,
RBC, Scanning Electron
Microscopy, SEM

Ethics clearance needs to be obtained before starting work. Check the laboratory has the required safety equipment and PPE for this protocol.

Coverslip preparation

- 1 Prepare glass coverslips by incubating them in **[TM] 10 % volume** poly-D-lysine solution in PBS for **🕒 00:30:00** at RT
- 2 Rinse twice with milliQ water
- 3 Dry and keep at **🌡️ 4 °C** until use

RBC fixation with glutaraldehyde

- 4 Wash **🧴 100 µL** of RBC in **🧴 900 µL** PBS (**🕒 00:02:00 at 1000g**)
- 5 Resuspend **🧴 25 µL** of the RBC suspension in **🧴 475 µL** of PBS

- 6 Add 500 μL of 2 % volume glutaraldehyde solution in PBS to the cell suspension slowly (drop by drop) to reach a final glutaraldehyde concentration of 1%

Safety information

Work under a fume hood when using glutaraldehyde

- 7 Incubate for 00:30:00 in the dark

- 8 Wash with 1 mL of PBS (00:02:00 at 1000g)

Adhesion on coverslip

- 9 Dilute 100 μL of fixed cells suspension into 400 μL PBS
- 10 Incubate 200 μL of diluted cell suspension on a poly-D-lysine coated coverslip (01:00:00) in corresponding buffer (in 6-wells plates)
- 11 Rinse slide 2 times with PBS (without scratching/damaging cell layer)

SEM sample preparation

- 12 Incubate in 0.1 Molarity (M) cacodylate buffer (00:10:00) . Repeat step 3 times

Safety information

Work under a fume hood during this section, check waste disposal for the different chemicals

- 13** Post-fix with [M] 1 Mass Percent osmium tetroxide (🕒 01:00:00)
- 14** Rinse twice with milliQ water (🕒 00:10:00)
- 15** Dehydrate the sample with ethanol
- Ethanol [M] 40 % volume (🕒 00:10:00)
 - Ethanol [M] 50 % volume (🕒 00:10:00)
 - Ethanol [M] 60 % volume (🕒 00:10:00)
 - Ethanol [M] 70 % volume (🕒 00:10:00)
 - Ethanol [M] 80 % volume (🕒 00:10:00)
 - Ethanol [M] 90 % volume (🕒 00:10:00)
 - Ethanol [M] 100 % volume (🕒 00:15:00)
 - Ethanol [M] 100 % volume (🕒 00:15:00)
- 16** Incubate in HDMS (🕒 00:30:00). Repeat step twice
- 17** Dry under the hood for 🕒 00:30:00 minimum (place filter paper under coverslip to prevent attaching to the culture plate)
- 18** Cover with a layer of gold palladium