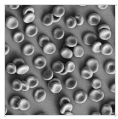

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Working

# Preparation of human Red Blood Cells for Scanning Electron Microscopy imaging

PLOS One

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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 µL 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 hexamethyldisilizane (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.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0215447

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Geekiyanage NM, Balanant MA, Sauret E, Saha S, Flower R, Lim CT, Gu Y (2019) A coarse-grained red blood cell membrane model to study stomatocyte-discocyte-echinocyte morphologies. PLoS ONE 14(4): e0215447. doi: 10.1371/journal.pone.0215447

MATERIALS

| NAME | CATALOG # | VENDOR |
|---|---|---|
| Glutaraldehyde EM Grade 25% | G5882-50ML | Sigma Aldrich |
| Ethanol 100% | | |
| MilliQ Water | | |
| PBS | 17-517Q | Lonza |
| Poly-D-Lysine | A3890401 | Thermo Fisher Scientific |
| Sodium cacodylate trihydrate | View | ProSciTech |
| Osmium Tetroxide | View | ProSciTech |
| Hexamethyldisilazane | View | ProSciTech |

MATERIALS TEXT

Fresh or stored red blood cells (RBC)

SAFETY WARNINGS

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

BEFORE STARTING

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

## Coverslip preparation

1 Prepare glass coverslips by incubating them in [M]10 Volume Percent 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 [M]2 Volume Percent 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 Rince slide 2 times with PBS (without scratching/damaging cell layer)

## SEM sample preparation

12 Incubate in [M]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 Percent ( 00:10:00 )
- Ethanol [M]50 Volume Percent ( 00:10:00 )
- Ethanol [M]60 Volume Percent ( 00:10:00 )
- Ethanol [M]70 Volume Percent ( 00:10:00 )
- Ethanol [M]80 Volume Percent ( 00:10:00 )
- Ethanol [M]90 Volume Percent ( 00:10:00 )
- Ethanol [M]100 Volume Percent ( 00:15:00 )
- Ethanol [M]100 Volume Percent ( 00:15:00 )

16 Incubate in HDMS ( 00:30:00 ). Repear 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