PROTOCOL FOR FIXATION OF ERYTHROCYTES
(fixed erythrocytes are used to detect pericellular coats)

- Materials: Refrigerated or frozen total blood w/anticoagulants
- Buffers: 10 mM PBS
  10 mM PBS w/ 10mM EDTA
  10 mM PBS w/ 1% glutaraldehyde (freshly prepared)

WARNING: THESE PROCEDURES USE TOTAL HUMAN BLOOD. BE EXTREMELY CAREFUL WITH ANY SOURCES OF CONTAMINATION AND FOLLOW ALL PROCEDURES TO AVOID BLOOD-BORNE PATHOGENS. PERSONAL PROTECTIVE EQUIPMENT IS MANDATORY!!

Anticoagulation tip: Before touching blood with tips or pipettes, use them to aspirate and discard a solution of 10mM PBS w/ 10mM EDTA

- Transfer 5ml total blood to a 15ml Falcon tube and centrifuge at 1000g x 10min (4C or room temp)
- Discard supernatant (plasma) and white layer of cells (buffy coat, leukocytes). The remaining packed cells are the erythrocytes (red blood cells, **RBCs**). The vol. of packed RBCs will be ~35-45% of the initial volume
- Wash RBCs three times in 10 ml of 10mM PBS. Cf 1000g x 10 min each time (4C or room temp). Remove large clumps and cloths manually and estimate the final volume of packed RBCs.
- Resusp RBCs in 10 volumes of 10mM PBS and chill on ice. Mix total volume of suspended cells with an equal volume of pre-chilled 10mM PBS containing 1% glutaraldehyde (final concentration glutaraldehyde: 0.5%). Transfer cells to a larger Falcon tube if the final volume is too large
- Incubate the RBCs in the cold room for 30-45 minutes, with end-over-end rotation. Centrifuge the RBCs at 1000g x 10min to remove the supernatant
- Wash the RBCs three times with cold 10mM PBS. Make sure to resuspend well the cells for each wash. Estimate visually the volume of packed cells at the end of the last wash.
- Resuspend the RBCs in 10 volumes of 10mM PBS.
- **(OPTIONAL:** count the RBCs in the hemocytometer. Cell density should be > 1.10^8 cells/ml)
- Add sodium azide to the RBC suspension (final concentration: 0.1% v/v) and store in aliquots at 4 C.
- For long term storage, add BSA in PBS to final concentration of 1 mg/ml and store in aliquots at -80C.
- Before using an aliquot, invert the tube several times and vortex cells briefly to make a homogeneous suspension, and remove any large clumps. Use these suspensions to detect hyaluronan coats, at dilution 1/10 to 1/100 in Hanks buffer or cell culture PBS.