Shear Stress induced Rolling of CD44 expressing Leukaemic Cells

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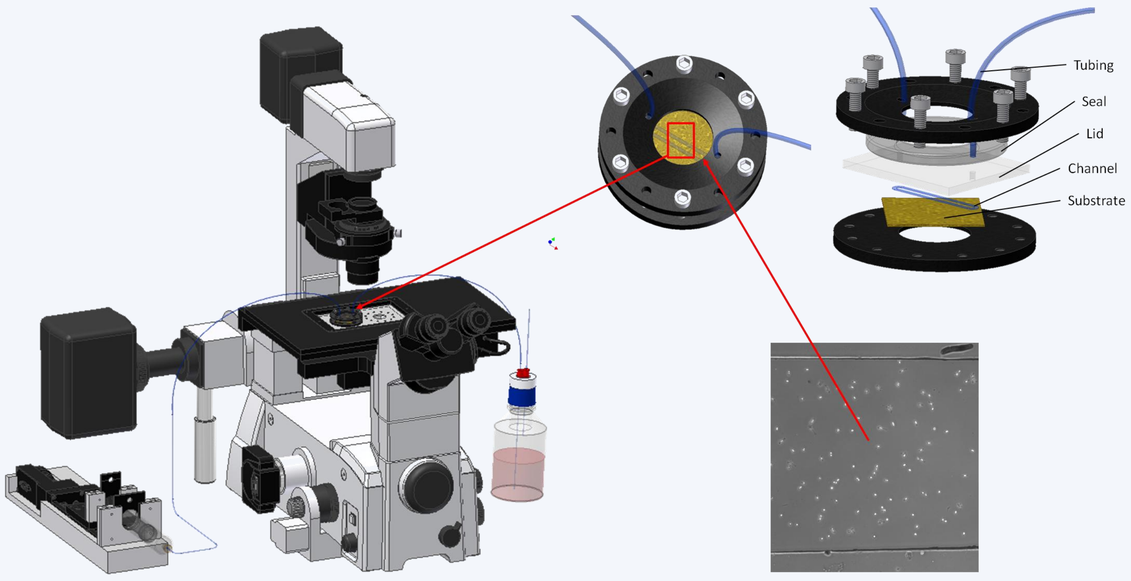
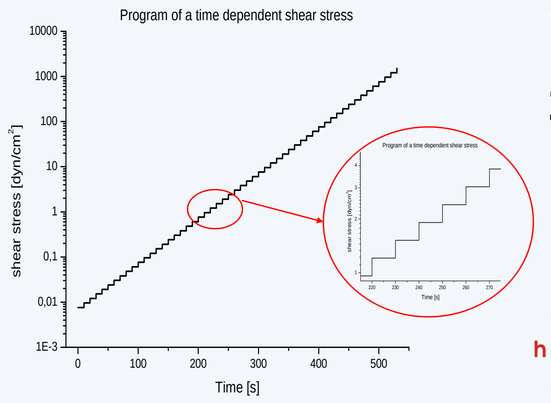
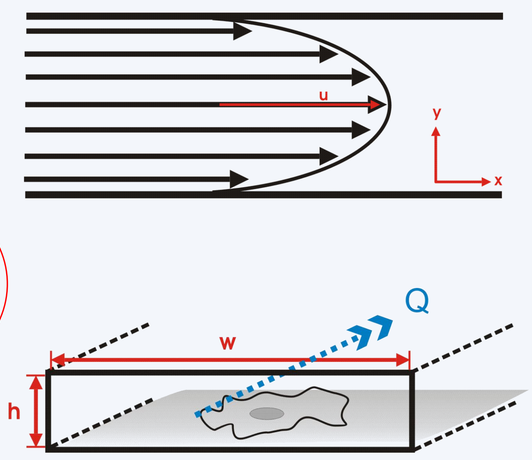
Introduction:

Cell adhesion is important as it affects the cell cycle, proliferation and differentiation. Studying the interaction of cells with artificial surfaces can serve as models for biological systems. In leukaemia therapy such knowledge is important as adhesion is connected with stem cell homing and thus with renewal of the hematopoietic system after leukemia therapy. Recent experiments under controlled microfluidic contidions[1] showed that leukaemic progenitor cell lines such as KG-1a and HL-60 are capable of rolling on Hyaluronan surfaces when a shear flow is applied.[1] This rolling phenomenon is similar to that of leukocytes in the extravasation process[2]. Antibody experiments reveal that binding of leukemic cells is CD44 and not selectin mediated. It could be shown that a maximum of cell adherence and thus activation of the catch bonds occurs at a shear stress of ~ 1dyn/cm².[1]



The setup: incubation microscope with syringe pump, microchannel and liquid reservoir.





$$\tau = \mu \frac{du}{dy} \quad (1)$$

$$\tau = \frac{6 \cdot \mu \cdot Q}{h^2 w} \quad (2)$$

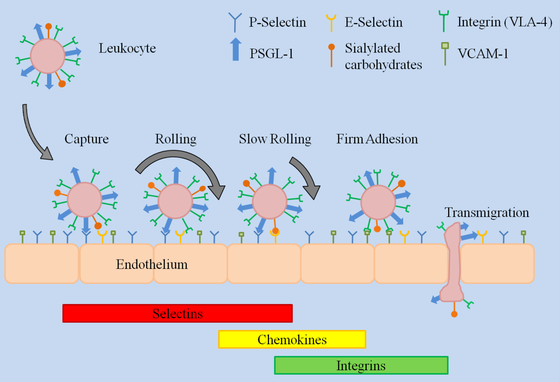
t = shear stress; μ = viscosity; *Q* = volumetric flow; *h* = channel height; *w* = channel width

Principle of hydrodynamic shear force in a parallel plate flow channel and flow program for the detachment experiment

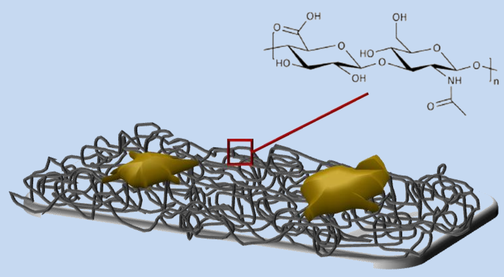
With this setup the adhesion strength, cell rolling speed and cell attachment rates can be studied. It is also possible to modify the surface properties to be able to study the surface s effect on cellular adhesion.

Cellular Rolling

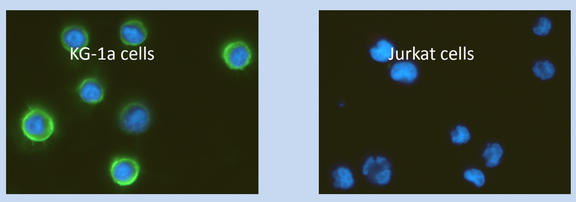
Leukocyte extravasation is a well known process. A similar rolling effect can be observed for cells, such as haematopoietic stem cells, expressing the receptor CD44 on hyaluronan surfaces.. KG-1a suspension cells proved to be a potent reference cell line for experiments adressing the importance of CD44 receptors for cellular rolling on hyaluronan surfaces.



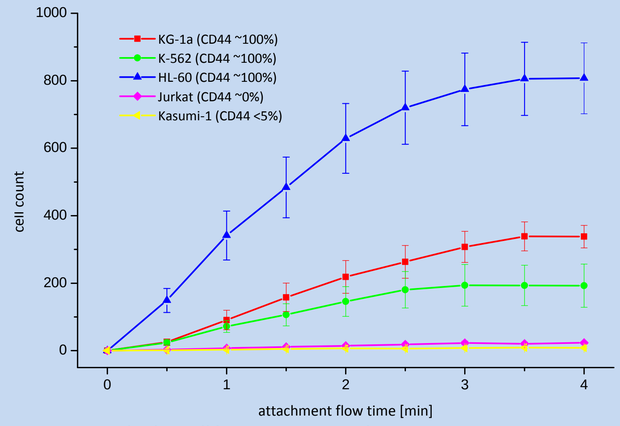
An important step in leukocyte extravasation is the rolling of the leukocyte across the endothelium. This phenomenon is selectin mediated.



The rolling of KG-1a cells is CD44 mediated, unlike leukocyte which is induced by selectins.



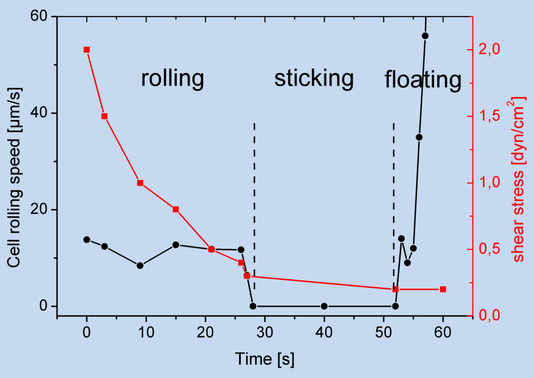
Blood carcinoma cell lines are suspension cells used as an alternative for hematopoietic stem cells to investigate the behavior of primary stem cells. Some types express the CD44 receptor (green stain), others don't.



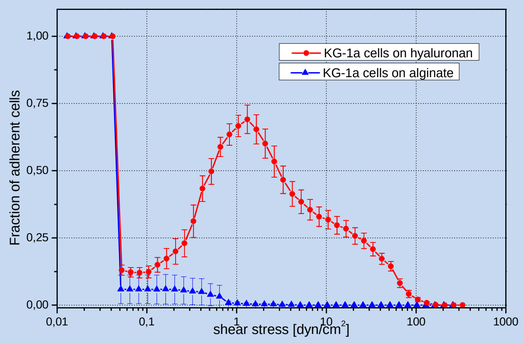
Only the cell lines expressing the CD44 receptor are able to attach from flow.

CD44 mediated Rolling and its Characteristics

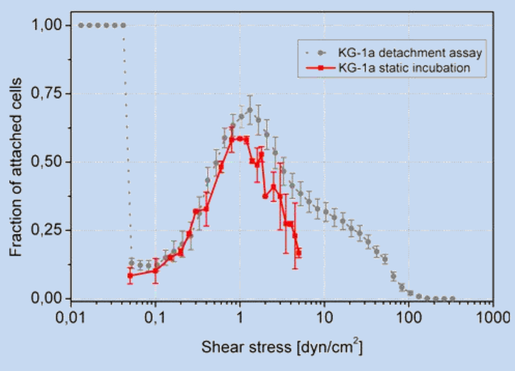
Accumulation and detachment assays are the two most commonly applied measurement techniques in our microfluidic system. They allow the calculation of cell rolling speed and the comparison of different cell lines or differently treated cells. The flow induced adhesion can be observed beyond a critical shear stress of ~ 0.2 dyn/cm² up to a maximum at ~ 1 dyn/cm².



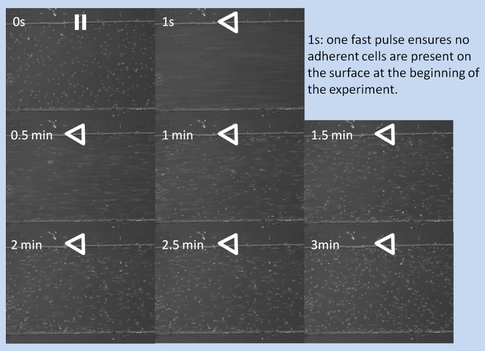
The rolling of cells expressing CD44 is flow induced and can only be observed beyond a critical shear stress.



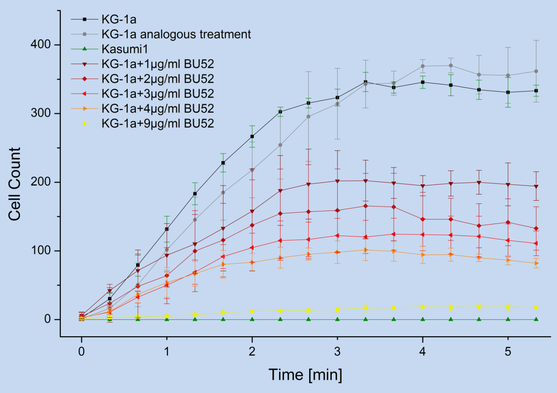
Cell adhesion strength assay: CD44 receptor expressing KG1-a cells barely stick on alginate but show flow induced cell adhesion and rolling on hyaluronan.



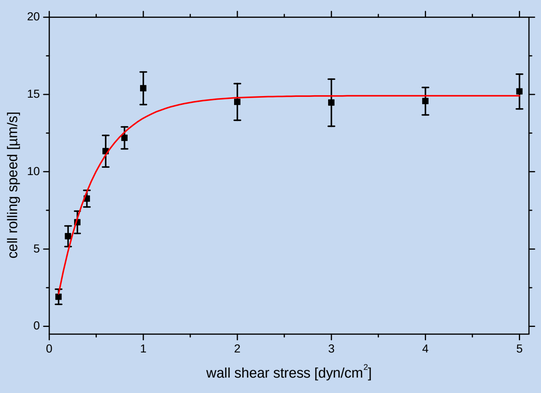
Two different techniques of measurement show the same results concerning the adhesion maximum of KG-1a cells on hyaluronan.



Accumulation assay: KG-1a cells accumulate on a hyaluronan surface at a constant flow of ~ 0.2 dyn/cm² (~ 0.00014 ml/min).



Attachment can be blocked by antibody blocking of CD44.



KG1-a cells respond actively to applied flow. The rolling speed reaches a maximum of ~ 15 µm/s at ~1 dyn/cm².

Conclusions

✓ Flow induced rolling of CD44 expressing cells can be observed after a critical shear stress of ~ 0.2 dyn/cm².
✓ Rolling can be suppressed by incubation with the antibody BU52 proving CD44 to be the receptor responsible for rolling.
✓ Rolling speed increases up to a maximum of ~ 15 µm/s at ~1 dyn/cm².

➢ Further studies of the CD44-Hyaluronan interaction, such as Hyaluronan modification.
➢ Analogous studies shall be performed with human material such as cord blood cells and haematopoietic stem cells to be able to compare their rolling characteristics.
➢ Behavior of stem cells under a chemokine gradient shall be studied to further understand the stem cell homing process.

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References

[1] C. Christophis, I. Taubert, G. R. Meseck, M. Schubert, M. Grunze, A.D. Ho, A. Rosenhahn, "Shear stress regulates adhesion and rolling of CD44+ leukemic and hematopoietic progenitor cells on hyaluronan.", *Biophysical Journal*, **2011**, **101**(1)

[2] K. Ley, C. Laudanna, M.I. Cybulsky, S. Nourshargh, *Nature Reviews Immunology*, **2007**, 678-689