

ADHESION OF CELLS AND MICROSPHERES IN A PARALLEL-PLATE FLOW CHAMBER

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INTRODUCTION: Cell adhesion under flow conditions has been extensively studied because of its important role e.g. in the immune and developmental system.

Since topographic cues have been shown to influence cell adhesion onto surfaces, our aim is to elucidate the mechanisms for the changes in adhesion onto nanometrically engineered surfaces. In particular, nanopillared surfaces have shown to be highly nonadhesive to living cells.

We will focus on the investigation of the interfacial forces of the nanopillared surface, by studying the interaction of charged microspheres and cells when flowing over the aforementioned substrate.

METHODS: A parallel plate flow chamber was built by melting a thermoplastic gasket (Sika Werke GmbH, thickness=150 μ m), between a glass slide and a solid PMMA sheet (Goodfellow Cambridge Ltd, thickness=250 μ m), the latter being the observed substrate.

The chamber is designed to create a laminar flow over the substrate: the flow is generated by a syringe pump, the syringe containing a suspension of cells or microspheres.

Phase contrast images were captured at 20X and 100X magnification, then digitized and analyzed.

The engineered PMMA is made by embossing a pillared pattern on the solid sheet. The pattern is written by electron beam lithography on a SiO₂ master, a nickel shim is then electroplated after the master, and used to emboss the polymer.

Carboxylated latex beads ($\phi=2\mu$ m) were purchased from Molecular Probes, Leiden, The Netherlands; and suspended in different NaCl solutions (approx. 10⁶ beads/ml).

Epitenon cells were suspended in ECT media, and the flow was kept for approximately 2 hours.

RESULTS: A parallel plate flow chamber was built, and its characteristics are shown fig.1

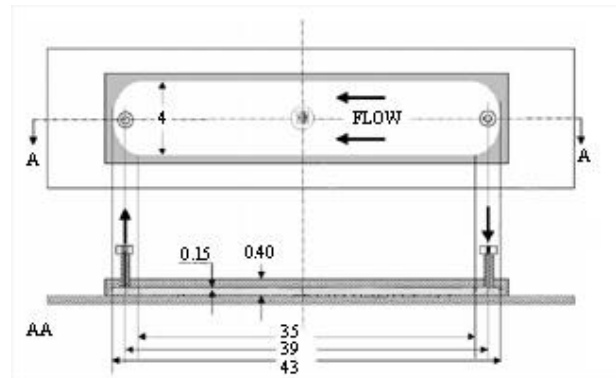


Fig.:. Parallel-plate flow chamber (from Pierres *et al.*, 1996)

X100 magnified images allowed to draw the trajectory and calculate the velocity of a single sphere near the surface, while X20 magnified images were used to count the average number of beads adhering onto a 60mm² area after 18 mins from the start of the flow .

DISCUSSION & CONCLUSIONS: This flow chamber has a certain number of advantages and drawbacks: its height is only 150 μ m, which helps having a creeping flow, it is very transparent and easy to assemble. Nevertheless, it can hardly be used more than once, the hot-melting sealing technique restricts the choice of materials, and it is hand-made, so the flow conditions are not exactly reproducible.

The variation of trajectory and velocity of the charged beads near the substrate is expected to give us some information on the interfacial forces acting on the beads; similarly, the variation of trajectory, adhesion and spreading of the cells could help us understand better the influence of nanopillared surfaces on cell binding.

REFERENCES: Curtis *et al.* (2001). *Biophys Chemi* **94**: 275-283. Doroszewski *et al.* (1988). *J Cell Sci* **90**: 335-340. Pierres *et al.* (1996). *J Immunol Meth* **196**: 105-120.

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