Fachtagung "Experimentelle Strömungsmechanik"

6. – 8. September 2016, Cottbus

Adhesion Limit of Fibroblasts Measured in a Narrow-Gap Rotational Rheometer

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Keywords Cell adhesion, critical shear stress, narrow-gap rheometry, cell detachment.

Abstract

The adhesion of cells to a substrate is crucial for implants and biofilms. As a test case, we study the adhesion limit of 3T6 Swiss mouse fibroblasts in a modified narrow-gap rotational rheometer in the parallel-disk configuration. The cells were fixed to the lower glass plate with the adhesion promoting protein fibronectin close to the rim of the gap between the disks. The critical shear stress was determined as the one at which half of the cells detach from the substrate. The critical shear stress depends on the fibronectin concentration and cell adhesion time. Maximum critical shear stress was achieved at 0.4 vol. % fibronectin concentration and an adhesion time of 60 minutes. Increasing concentration or adhesion time did not result in higher critical shear stresses.

Introduction

Shear-induced detachment and displacement of solid or soft particles is encountered in countless environmental, industrial, biological and medical systems. Recently, we showed for solid particles that critical conditions do not depend on particle inertia but may be influenced by neighbors (Agudo, et al. 2014; Agudo and Wierschem 2012). In this contribution, we focus now on the adhesion of soft biomaterials.

Biomaterials have been used as long-term implants in medical, dental, veterinary and pharmaceutical fields. Biocompatibility plays an important role for the success of an implant. One important aspect is the cell adhesion to the surface of the implant. The absence of cell adhesion can lead to infection, loosening or wear of the biomaterial (Yildirim 1998). While the adhesion of blood cells can be fatal to a vascular prosthesis, an anchoring of hip joints is not only desirable in the bone, but also required (Yildirim 1998).

The cell adhesion to a substrate is a complex process, which still leaves many opened questions. Various methods have been developed to investigate the rheological properties of cells. They are usually divided into passive and active methods. Passive methods do not apply forces to the cells to be examined. As an example: A method for assessing the deformability of a cell is sucking in a very small micropipettes. By a predetermined constant diameter of the tip pressures of up to 10^5 Pascal can be generated. This method is used in determining the mechanical properties of red blood cells, because their cytoskeleton is built relatively easy and

Karlsruhe, Germany, ISBN 978-3-9816764-2-6

sticks tightly to the cell membrane. For more complex cells such as fibroblasts, however, this method is not viable (Verdier, et al. 2009).
Fibroblasts are mobile cells, which can be found in the connective tissue. Their main task is the continuous production and maintenance of the extracellular matrix, a fibrous network of proteins and carbohydrates. The containing structure protein collagen for example ensures the stability in the body. If injury or inflammation occurs, fibroblasts start to migrate to the required place and proliferate to enhance collagen production, which is essential for the wound healing processes (Grinnell 2003). In response to their environment, fibroblasts can change shape and size ratios. In contact with a substrate, suspended spherical fibroblasts first start to adhere and then they spread on the substrate taking accordingly a flat and unfolded shape. The adhesion strength depends on the material, the intracellular structure and the surrounding cell density. Like all other cell types, fibroblasts are aging. This increases their stiffness by up to about 60% (Schulze, et al. 2010).
Cells adhere to surfaces via certain proteins like fibronectin, laminin, collagen and GMEM (Lotz, et al. 1989). For instance, Engler et al. found an eight-fold increase of the required shear stress for detaching human fibroarcoma cells from a fibronectin coated glass substrate (Engler, et al. 2009). Fibronectin is composed of two linear chains, which consist of three repetitive subunits. These two chains are linked by two disulfide bridges. Using a carboxyl group, fibronectin can bind to a cell, while an amino group allows bonding to other surfaces at the other end (Couchman, et al. 1983). Adhesion is promoted by enhancing interactions of fibronectin with the receptors on the surface of the cell. These receptors are called integrins (Dee, et al. 2002). According to the signal distribution of the integrins, a cell can change its shape or stimulate the production of proteins like fibronectin, which yields to focal adhesion.
We studied shear-induced cell detachment from a flat glass plate covered with a fibronectin film. To set up and control a well-defined shear flow even at high shear rates, we employed a narrow-gap rotational rheometer in the parallel-disk configuration. The critical shear stress for cell detachment was defined as that at which half of the cells detach from the plate. The considerable improvement in precision of the narrow-gap rotational rheometer compared to commercial rheometers allows studies at gap widths of the order of about 10 µm (Dakhil and Wierschem 2014).

Materials and methods

The experiments were performed on 3T6 fibroblasts, which have a typical size of about 7 µm, cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS), 15 mM HEPES and $NaHCO_3$, Pyridoxine and L-glutamine (all from Sigma-Aldrich). Cells were cultured at 37°C and 5% CO_2 according to standard procedures and were passaged weekly. The cells are prepared for the rheological experiments in same procedure as in former studies. For details we refer to (Dakhil, et al. 2016).

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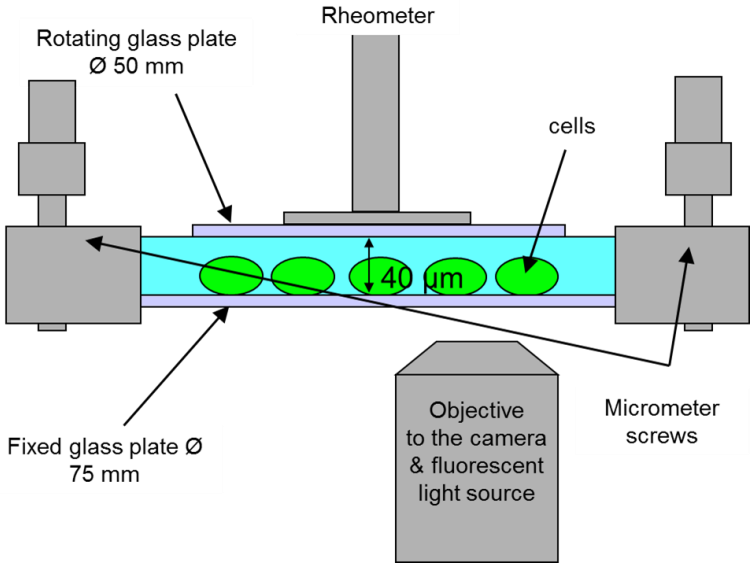

Fig. 1: Sketch of the setup in the customized rheometer. The gap width between the rheometer plates is measured with a confocal interferometric sensor; the cells are viewed with a camera.

A customized rotational rheometer (UDS 200, Physica) was used in all experiments (see Fig. 1 for schematic drawing). The disks of the device are designed from circular glass plates (Edmund Optics). The lower plate (diameter: 75 mm; evenness: λ/4; λ: 633 nm) is fixed whereas the upper plate (diameter: 50 mm; evenness: λ/10) is designed to rotate. The top plate is glued to a measurement head of the rheometer (diameter: 25 mm). To align the bottom plate perpendicularly to the rotation axis, it was fixed to a tripod that was mounted to the rheometer table. The tripod was aligned with three micrometer screws and fixed to the rheometer with three screws after adjustment. With this setup, the gap width can be set up with a precision of about ±0.7 µm; fixing the relative phase of the two plates allows further improvement of the precision. To retain this accuracy, the rheometer was recalibrated once a day following a procedure described previously (Dakhil and Wierschem 2014).

For bright field and fluorescence microscopic imaging of cells, the rheometer is equipped with a modular and customized assembled fluorescence microscope (Thorlabs) installed in the housing of the device. Primary resolution of the camera was 1280×1024 pixel. For imaging cell detachment, it was equipped with a 5x objective lens (N PLAN 5x/0.12, LEICA). Further details on the optical setup are provided in (Dakhil, et al. 2016).

Before the experimental runs, the glass plates of the rheometer were coated with a fibronectin solution (Sigma-Aldrich). The solution was made by adding fibronectin to phosphate buffer solution (PBS). The final fibronectin concentrations were between 0 and 1 vol. %. After 1 hour, the solution was removed. Only a thin fibronectin film remained fixed to the glass plate. Thereafter, the cell suspension was introduced between the glass plates. It was left for 20 minutes to allow the cells to sediment and to attach to the lower plate before the upper plate was moved towards the lower disk at minimum speed at a gap width of 40 µm. In this configuration, the cells were left for 1 hour to allow for improved cells adhesion. The experimental runs were performed as stress-controlled tests covering the range between 0.1 Pa and 100 Pa. 40 measurement points were taken with 20 seconds per data point in a logarithmic ascent.

Experimental results and discussion

A typical example for cell counting procedure shows Fig. 2 with individual cells and group of cells, where cells are in direct contact with each other. But also two cells were declared as a group if their distance is less than three times their size. This discrimination was set as in the former studies (Agudo, et al. 2014; Agudo and Wierschem 2012). Single cells show less resistivity than cell groups and detached faster. However, the size of the group does not show any differences in the required shear stress to detach them.
Fig. 3 shows the percentage of detached cells as a function of shear stress. In this figure, the cells were seeded on an uncoated glass plate and stress was varied between 0.1 Pa and 1.1 Pa. At the stress of 0.1 Pa, the first cell was detached from the lower plate. The number of detached cells continuously increases with the shear stress and at 0.6 Pa all the cells were detached. The circles show an experimental run where the cells were seeded on a glass plate coated with 1vol. % fibronectin. In this case, the first cell detached at a shear stress of 7 Pa. In contrast to the uncoated case, the detachment did not increase continuously with shear stress: In this particular case, the cells were detached in small groups at stresses of 3 Pa, 20 Pa, and 28 Pa. As shows Fig. 3, fibronectin increases the critical shear stress for detachment by one order of magnitude.

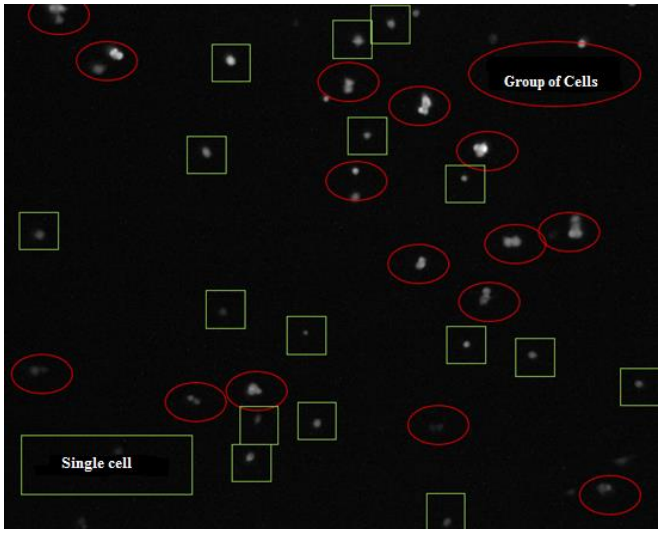

Fig. 2: Single cell and groups of cells in direct contact with each other.

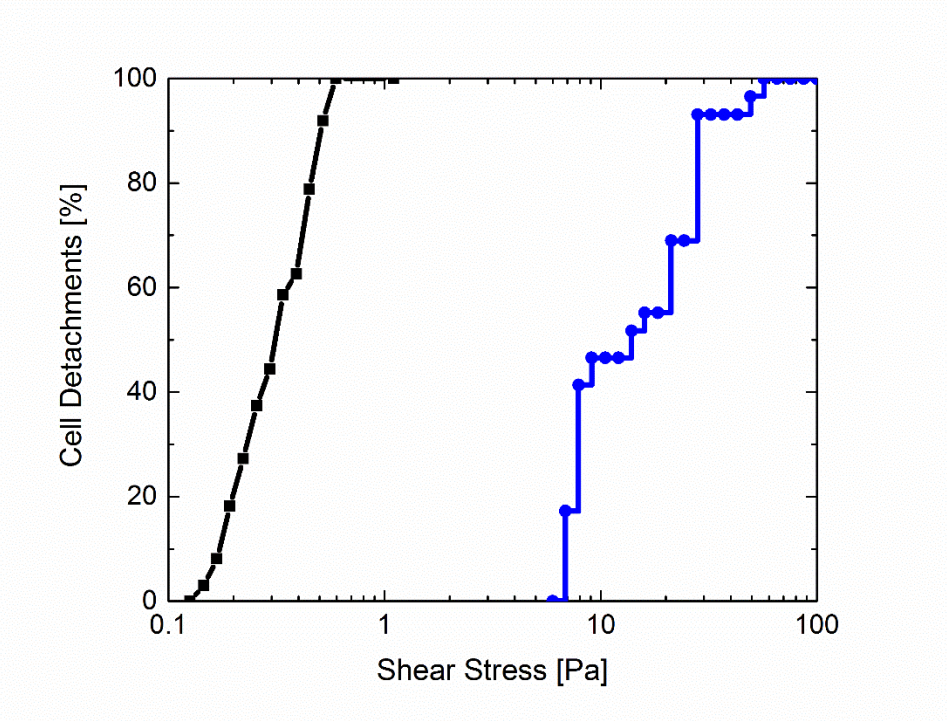

Fig. 3: Stress-controlled test with uncoated lower glass plate (cubes) and with the plate coated with 1 vol. % fibronectin protein (circles).

Conclusions

Cell adhesion to biomaterials plays crucial role in biofilms and in implants. We used a narrow-gap rotational rheometer in the parallel-disk configuration at gap widths of 40 µm gap width and detect the cell detachment optically. We determined the critical shear stress of 3T6 fibroblast cells that needs to detach half of the cells from their connected substrate.

Acknowledgments

This work was supported by grants from Deutscher Akademischer Austauschdienst (DAAD), Germany and the Ministry of Higher Education & Scientific Research, Iraq. We thank the workshop members at the Institute of Fluid Mechanics for their advice in building the setup.

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