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Nanomedicine: possible early diagnostics of leukemia via atomic force microscopy of red blood cells

Ille C. Gebeshuber[1,2,3,4], Michael Haidinger[5], Christoph G. Goesselsberger[3], Eva Wollmann[5] and Oliver Hekele[3]

[1]*Institute of Microengineering and Nanoelectronics (IMEN), Universiti Kebangsaan Malaysia, Malaysia, ille.gebeshuber@ukm.my*

[2]*TU BIONIK Center of Excellence for Biomimetics, Vienna University of Technology, Austria*

[3]*Institut fuer Allgemeine Physik Vienna University of Technology, Austria*

[4] *AC²T Austrian Center of Competence for Tribology, Austria*

[5] *Medical University Vienna, Austria*

Inspired by the abuse of erythropoietin (EPO) in serious sports (doping) it was investigated in a previous study if there is a difference in the structure and stiffness of red blood cells which are produced due to synthetic EPO and body own EPO [1][2]. No statistically significant difference in the stiffness of red blood cells could be detected between the treated and the untreated group. In course of these measurements, a significant deviation of the stiffness of one of the patients of the control group was detected. The red blood cells of this patient were about four times less stiff than the ones of the other patients investigated (Figure 1) and the red blood cells also did not have the typical donut shape, but were oblate like a coin (Figure 2). After more detailed medical investigation a rare case of diabetes was diagnosed in this donor in 2007.

Now, two years after the study, this patient with the abnormal red blood cells is treated for chronic myelotic leukemia and is currently, three months after the start of the therapy, in clinical remission. Since malign diseases of the blood are known to also yield deformation of the red blood cells, the authors are currently investigating changes in erythrocyte morphology after therapy, using atomic force microscopy.

I. Atomic Force Microscopy for Investigation of Red Blood Cells

There are several reasons for using the atomic force microscope (AFM) in studying blood cells; the most important is that the AFM is a general-purpose instrument for analyzing surfaces at ultrahigh resolution, in ambient, fluid or vacuum conditions. Compared to other analytic instruments the AFM and especially the ambient AFM has a variety of advantages. The main advantage of this method is that non-conducting samples can be analyzed without additional preparation such as metalizing with gold or similar techniques.

For the experiments in the study the stiffness of blood samples of renal insufficient patients (i.e. patients with kidney problems), who were medicated with synthetic EPO and blood samples of a control group (healthy individuals) were compared [1][2]. The maximum age of the donors was 50 years.

After the standard procedure of preparation of the blood samples [3], the cells were imaged with the AFM using dynamic mode. Subsequently AFM force vs. distance curves were recorded and evaluated for differences in penetration depth. The same method is applied again in the current study.

The images are recorded in dynamic mode to prevent damaging the sample by scratching over it, with cantilevers with 70 kHz resonance frequency in air and a spring constant of 1.8 nN/nm (Olympus OMCL-AC240TS). The scan frequency is 0.43 Hz (i.e. a little bit less than two lines per second), the image size is originally 20x20 μm^2, regions of interest are subsequently scanned with smaller scan size.

For the question if there is a difference in the stiffness and plastic deformability of the control group and EPO medicated patients, the most interesting parameter is the penetration depth. The penetration depth is a parameter for the deformability of the cells. It is evaluated by the position of the maximal movement of the piezo sensor in z-direction, which is the coordinate when the sensor reaches the trigger point of 3 µN, minus the position of the sensor when the tip is in first contact with the surface of the red blood cell.

After imaging the erythrocytes, force spectroscopy with trigger forces of three micronewtons is performed on each single cell along preset paths in ambient air. In the first study, about 200 force vs. distance curves were recorded per sample, and 25 samples were investigated [1][2].

The penetration depth did not reveal statistically relevant differences in healthy and EPO blood samples. However, the penetration depth of samples 10 and 11 (who came from the same donor) was four times higher than the penetration depth of the other samples (see Figure 2).

There were also abnormalities of the surface of the blood cells of the donor of samples 10 and 11. The erythrocytes did not have the typical donut shaped form. These blood cells were oblate and very flat. The measurements were repeated in order to ensure that no destruction of the sample during transportation or preparation took place. The result was confirmed, the cells were again oblate and flat and the penetration depth of the cantilever was four times higher than in the other samples, indicating softer cells. After more detailed medical investigation a rare case of diabetes was diagnosed in the donor of samples 10 and 11 in 2007.

The AFM successfully proved as a nanodiagnostic tool. Minamitani and co-workers measured the deformability and viscoeleasticity of erythrocytes of patients with Diabetes mellitus, the common type of diabetes, by microchannel flow systems and atomic force microscopy. The blood cells of the patients with diabetes mellitus are harder than the control group [4][5]. The reason for the softening of erythrocytes in the rare case of diabetes presented here and the hardening of erythrocytes in diabetes mellitus is yet to be determined. Erythrocytes of people with diabetes also have a shorter live span compared to erythrocytes of a healthy control group. Currently, diabetes is diagnosed solely via chemical methods.

II. CURRENT STUDY AND OUTLOOK

Since now this patient is treated for chronic myelotic anemia and since malign diseases of the blood are known to yield deformation of the red blood cells, the authors currently perform the same experiments on the blood cells of this donor, to investigate any changes in erythrocyte morphology after therapy. These studies might provide first evidence for the applicability of atomic force microscopy based methods in the early diagnostics of leukemia and might lead to a fast and cheap screening method for early leukemia diagnostics.

Acknowledgment

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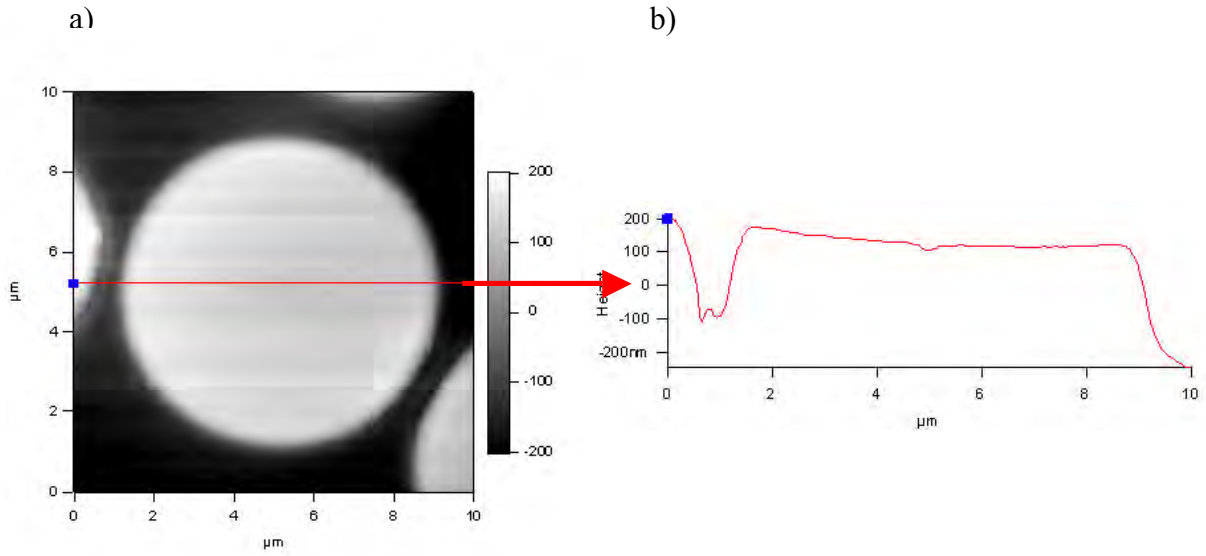

Figure 1. Abnormal red blood cell: a) Height trace b) Height profi1e.

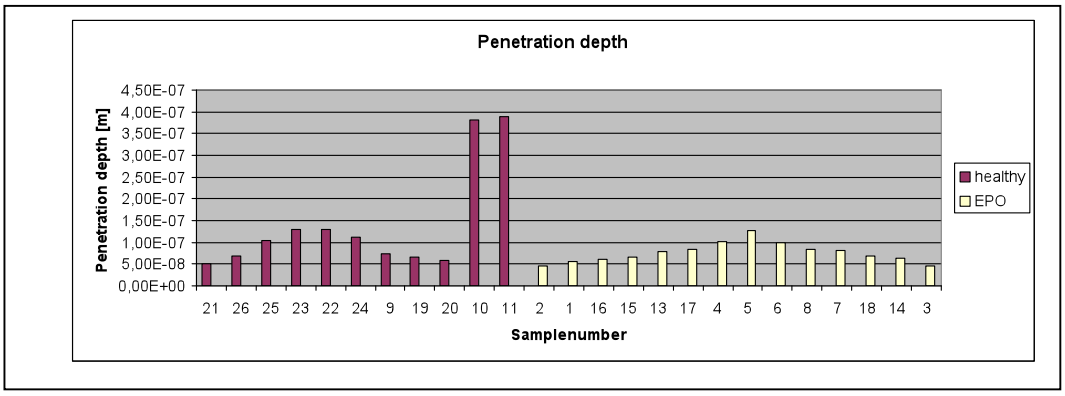

Figure 2. Penetration depth of all 25 blood samples.