Biopsy on living cells by ultra slow instrument movement

2006 J. Phys.: Condens. Matter 18 S627

(http://iopscience.iop.org/0953-8984/18/18/S09)

INSTITUTE OF PHYSICS PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **18** (2006) S627–S637 doi:10.1088/0953-8984/18/18/S09

Biopsy on living cells by ultra slow instrument movement

Hagen Thielecke, Impidjati and Günter R Fuhr

Fraunhofer-Institut für Biomedizinische Technik, Ensheimer Strasse 48, 66386 St Ingbert, Germany

E-mail: hagen.thieleckeh@fraunhofer.ibmt.de

Received 30 July 2005, in final form 7 February 2006
Published 19 April 2006
Online at stacks.iop.org/JPhysCM/18/S627

Abstract

Biotechnology and regenerative medicine require more and more nondestructive and gentle single cell characterization and manipulation. We surmise here that such cell manipulation can be gently performed by the ultra slow movement ($<50\ \mu m\ h^{-1}$) of a probe or recovery tool. This extremely slow instrument velocity is adapted to the natural cellular processes of cytoskeleton and membrane reorganization. Here we describe for the first time the following surprising effects, which occur as a result of ultra slow instrument motion. (i) Parts of individual cells enveloped by the plasma membrane can be cut from the cell body without destroying the cell. Both parts migrate over hours and the part with the nucleus continues its normal cell migration and growth. Separated parts from cells can be used for immunohistochemical staining. (ii) Two adherently growing and partly overlapping cells can be fused by ultra slow instrument movement through the overlapping cell region. (iii) Relatively large instruments penetrating the cell membrane can be moved inside a cell without damage. (iv) The direction of cell migration can be controlled by ultra slow tool procedures. It thus seems that extremely slow instrument motion is potentially a new manipulation technique for *in vitro* culture and opens up a variety of handling devices.

M This article features online multimedia enhancements

(Some figures in this article are in colour only in the electronic version)

1. Introduction

The past decades have seen advanced techniques for the manipulation of cells. Prominent examples are laser-based optical tweezers, the electromanipulation of cells like electrofusion and handling in dielectrophoretic fields [1–5]. However, biotechnology and regenerative

medicine require more and more nondestructive and gentle characterization and manipulation of single adherent cells. For complex characterization and to be available for any analysis, it would be the best to cut part of a cell without influencing its viability for biochemical, genetic or other characterization (single cell biopsy). We surmise here that such cell manipulation can be gently performed by ultra slow movement (<50 μm h^{-1}) of a probe or recovery tool. This extremely slow instrument velocity is adapted to the natural cellular processes of cytoskeleton and membrane reorganization. Recently, we demonstrated the possibility of using ultra slow velocities to separate selected single cells from surface cell layers *in vitro* without biochemical treatment or mechanical stress [6]. Tools moving as slowly as cells normally migrate can achieve gentle single cell handling. The migration velocities of animal and human cells lie in the range of μm h^{-1} to hundreds of μm h^{-1}. This is extremely slow and corresponds to the reorganization of the cytoskeleton and the repair mechanisms of the cell membrane system. We have shown that it is possible to move a probe ultra slowly (25 μm h^{-1}) through a monolayer of cultured cells or into a three-dimensional cell aggregate without causing damage [6]. Here, we report various other manipulations on cells and some remarkable effects which can be achieved with ultra slow probes smaller than cells. The following effects of manipulation using ultra-slow tools were observed.

- Samples of cytoplasm enveloped by intact plasma membrane can be cut and removed without destroying the cell. The separated parts can be used for immunohistochemical staining.
- Two partially overlapped, adherently growing cells can be fused by ultra slow instrument movement through the overlapping cell part.
- Micro instruments can be moved for hours inside a cell without causing damage, which opens up new applications with intracellular sensors.
- The direction of migration of a cell can be controlled by ultra slow manipulation of glass tools.

2. Material and methods

2.1. Estimation of the required velocity of tools

The manipulation tool must move so slowly that the cytoskeleton and membrane system of the target cell can rearrange in a natural way. Generally, this means probe velocities less than that of cytoskeletally controlled locomotion. The data used for the estimation of instrument velocities are based on investigations into the dynamics of cell adhesion and cell trace production by migrating animal cells *in vitro* [7]. Individual cells rearrange their cytoskeleton on a timescale of minutes to hours. We therefore wanted an instrument to generate speeds of tens of μm h^{-1} or a few nm s^{-1}.

2.2. Experimental setup for cell manipulation

Cell layers and aggregates on glass surfaces were manipulated using different tools under microscopic observation (see figure 1). The tools were attached to a computer-controlled micromanipulator and could move in all directions with freely selectable velocities. As there is no commercially available micromanipulator capable of producing continuous motion in this range, we used a stepper motor-based system giving stepwise movement with a step length of 40 nm. The manipulator was an Eppendorf (type TransferManNK2, Hamburg, Germany) with self-developed control software capable of 40 nm steps and with velocities from tens of

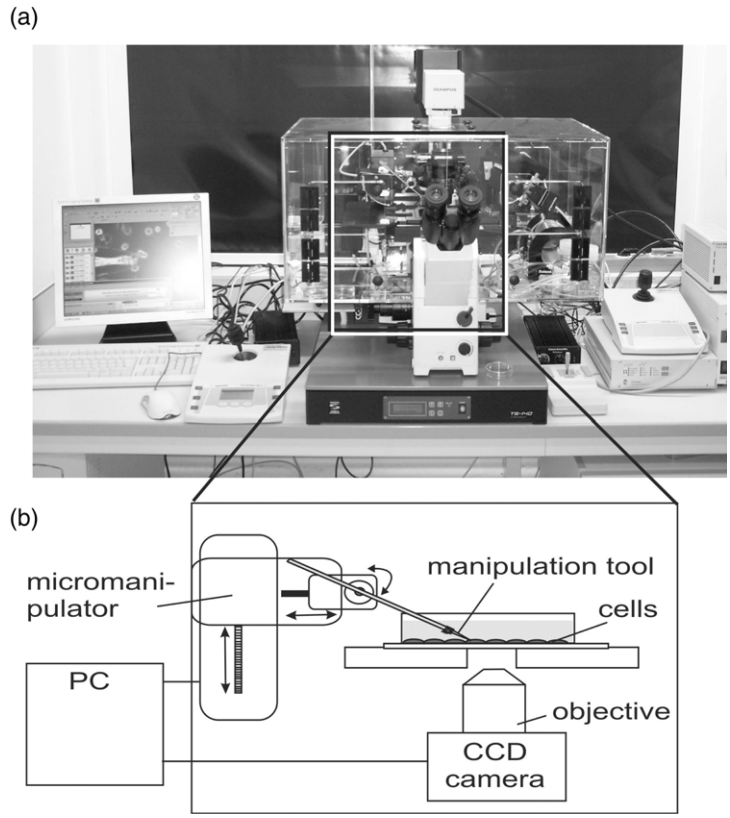

Figure 1. Experimental setup. (a) Photograph, (b) simplified schematic diagram.

μm s^{-1} down to a few μm h^{-1}. A control program for velocities in the range of 1 nm s^{-1} was developed using LabView (National Instruments, München, Germany). The temperature of the culture medium was adjusted to 37 °C using an incubator system (Solent Scientific). Phase contrast images were recorded at intervals between 1 and 30 s with a type IX71 microscope (Olympus, Hamburg, Germany) and a CDD-camera (type F-View, Soft Imaging System, Münster, Germany) using the image acquisition software analySIS 3.2 (Soft Imaging System, Münster, Germany). To reduce the effect of vibrations from the environment we placed the microscope on a TS140 vibration isolation system (HWL Scientific Instruments, Ammerbuch, Germany).

2.3. Tools for cell manipulation

For the cell manipulation we used tools made of glass. These glass tools were borosilicate glass capillaries (type 150-3, WPI, Sarasota, USA) pulled automatically using the microprocessor-controlled puller (type Pul-100, WPI, Sarasota, USA) to a tip diameter less than 1 μm. In the experiment performed to demonstrate the control of cell migration by ultra slow tools we also used larger tip diameters. Tips were flattened using a beveller (type 1300M, WPI, Sarasota, USA) and lapped with a micro forge (type MF-200, WPI, Sarasota, USA) under a microscope. Before use the glass tools were cleaned with isopropanol and deionized water in an ultrasonic bath.

2.4. Cell culture

The experiments were carried out with different cell types. We used L-929 cells, spontaneously differentiated adult stem cell from rat pancreas [8] and human macrophages [9, 10]. The mouse fibroblast L-929 cell line was obtained from the DSMZ-Collection of Microorganisms and Cell Cultures (Braunschweig, Germany), cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 50 units ml^{-1} Penicillin and 50 $\mu g\ ml^{-1}$ streptomycin (medium and supplements from Invitrogen, Carlsbad, CA, USA) at 37 °C in a humidified incubator gassed with 7.5% CO_2 for at least one day, then harvested and dispensed onto a cell culture treated glass slide with polystyrene vessel (Falcon CultureSlide type 4102, Becton Dickinson, Le Pont De Claix, France). The culture procedures of the other cell types were according the references cited above.

2.5. Cell viability test

Cell viability was assessed using a two-colour fluorescence-based method using fluorescein diacetate and propidium iodide (both from Sigma-Aldrich, Seelze, Germany). Fluorescein diacetate is a fluorogenic esterase substrate that is hydrolysed intracellularly to a green fluorescent product. Propidium iodide is a high-affinity, red fluorescent nucleic acid stain that is only able to pass through the compromised cell membranes. Stock solutions were 5 mg ml^{-1} fluorescein diacetate in acetone and 2 mg ml^{-1} propidium iodide in water and the working solution was obtained by diluting 200 μl (propidium iodide) and 60 μl (Fluorescein diacetate) of the stock solutions in 10 ml PBS. For staining, the cell culture medium in the vessel of the culture slide was replaced by 1 ml working solution. After 15 min incubation, the working solution was removed and the cells were washed once using PBS. Cells in PBS were examined under a fluorescence microscope (type B2-RFCA, Olympus, Hamburg, Germany).

3. Results

In a first series of experiments, we moved a glass probe with a tip diameter of 300 nm through cells cultured on a conventional fibronectin coated glass surface (see video 1 online available on stacks.iop.org/JPhysCM/18/S627).

For the experiments cells from the L-929 cell line and adult stem cells isolated from acini of rat pancreas were manipulated. The glass tip was pressed moderately against the glass substrate during movement (angle to surface 45°). Figure 2 compares the results when the tip was moved faster than 7 $\mu m\ s^{-1}$ (a typical speed for conventional techniques such patch-clamping, microinjection, biopsy techniques and cell transfer) and at ultra slow velocities of 7 $nm\ s^{-1}$ ($\approx$25 $\mu m\ h^{-1}$). At the higher speed the cells were either pulled from the surface or cut by the tool (figure 2(b)). Cell destruction and escape of cytoplasm was observed. In contrast, at ultra slow speed parts of a cell can be separated without destruction (figure 2(c)). After cutting, the nucleated part (figure 3(b)), as well as the part without nucleus (figure 3(c)), actively migrate, show cytoskeletal activity and both are always enveloped by plasma membrane (see video 2 online available on stacks.iop.org/JPhysCM/18/S627). The activity of the part without the nucleus stops after a few hours, while the nucleated fragment behaves like the unmanipulated, neighbouring cells. We performed 15 creep separation experiments. In all cases the separated part of cell as well as the nucleated part showed cytoskeleton activity after the creep separation procedure. After creep separation fluorescence staining using Fluorescein diacetate and propidium iodide showed the integrity of plasma membrane and enzymatic activity in cytoplasm of the nucleated part as well as of the separated part without nucleus (figure 4).

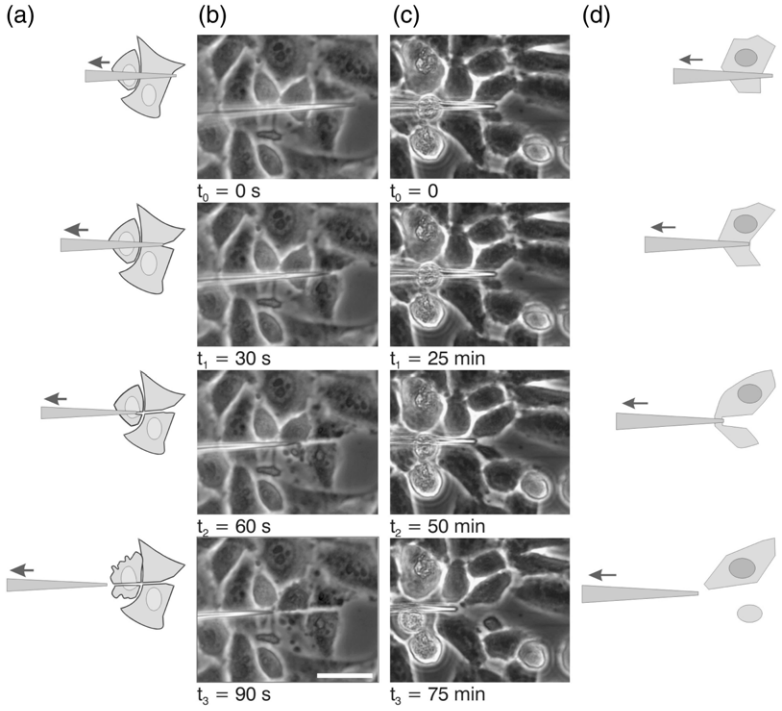

Figure 2. The effect on fibroblasts L-929 cells of rapidly moved (left row) and extremely slowly moved (right row) thin glass tips. ((a), (d)) Schematic drawings of the fast (a) and ultra slow (d) operation modes. (b) Cutting off of L-929 cells by a rapidly moved glass with a diameter of 300 nm leads to complete damage of the treated cell. (c) In contrast, the ultra slowly moved (7 nm s^{-1}) glass tip leads to separation of a small part of a L-929 cell from the cell body with nucleus. A reorganization of cytoskeleton and the membrane occurs during the slow manipulation and results in vital cell parts both migrating over the surface (Bar = 30 μm).

In a next experiment we moved the tip of a glass rod (angle to surface 45°) with a speed of 7 nm s^{-1} through two L-929 cells in a region where they overlapped (figure 5). At ultra-slow instrument velocities there was mechanically induced fusion (see video 3 online available on stacks.iop.org/JPhysCM/18/S627). Again, all parts of the cells were enveloped by intact plasma membrane. The fused cells were vital and normal active migration was observed for hours after the manipulation (figure 5(c)). The procedure and fusion could be repeated with different cell pairs.

In a third series of experiments we moved a glass tip with a speed of 7 nm s^{-1} (7 nm $s^{-1} \approx$ 25.2 μm h^{-1}) through a human macrophage cultured on a glass surface (angle to surface 45°) (figure 6). The cytoplasm membrane was penetrated by the tip but streamed around the instrument. At such slow velocities the tip could be moved inside the cell for hours during active migration of the cells on the surface of the cell culture system. No cell damage was observed and normal migration and cytoplasmic activity of the cells was seen during tip penetration.

In a last group of experiments we investigated the control of cell migration by ultra slowly moved instruments. First an uncoated glass probe of 10 μm diameter (comparable to the size of the cell) was moved with a speed of 7 nm s^{-1} ($\approx$25 μm h^{-1}) towards a stem cell cultured on a glass surface. In response to repeated touches by the probe, the cell actively moved away (i.e. in the direction of probe travel). In a further experiment we used a glass tool with a tip diameter

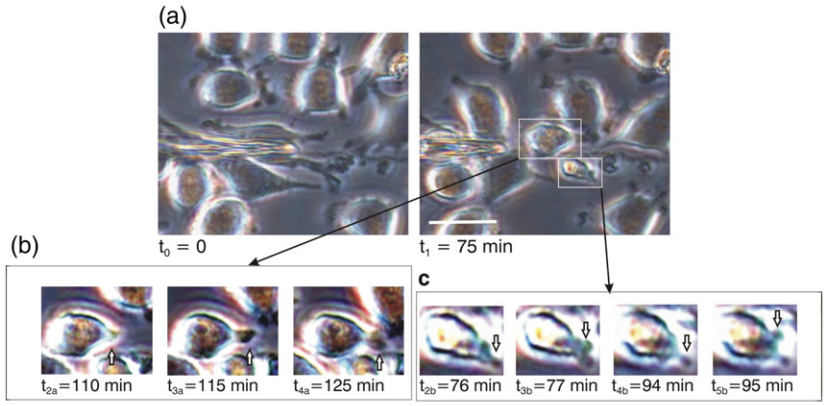

Figure 3. (a) Cutting parts of a L-929 cells enveloped by the plasma membrane from cell body without destroying the cell (Bar = 30 μm). (b) Sequences of phase contrast images of the cell after the manipulation. The arrows indicate the cytoskeleton activity. (c) Sequences of phase contrast images of the separated part without nucleus. The arrows indicate the cytoskeleton activity (filopodia formation).

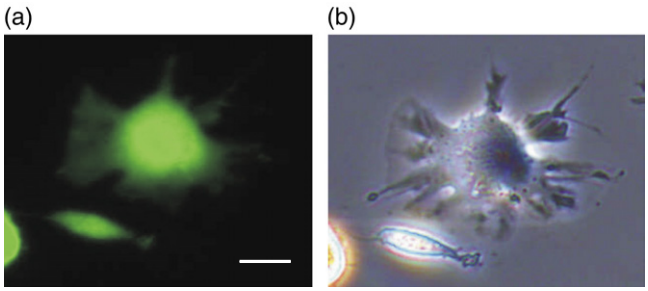

Figure 4. Test of plasma membrane integrity and enzymatic activity in the cytoplasm of a separated cell part and of the nucleated cell by fluorescence staining using Fluorescein diacetate and propidium iodide (FDA-PI) after creep separation. (a) Fluorescence image of the nucleated cell and of the separated cell part. With FDA-PI, live cells stained bright green and nonviable cells were bright red (Bar = 10 μm). (b) Corresponding phase contrast image.

of less than 1 μm. The glass tool was pressed on the glass surface until the tip bent up slightly. Between the tip and the glass surface there was a distance of about 1–5 μm. The tip was moved to an adherent isolated L-929 cell. The cell adhered on the top side to the glass tool while keeping the adhesion to the surface of the culture dish. Afterwards the tip was moved with a speed of 7 nm s^{-1} towards a group of cells. The cell migrated together with the ultra slowly moved tip until the cell was surrounded by a group of cells (figure 7, video 4 online available on stacks.iop.org/JPhysCM/18/S627). After interaction with other cells the cell released its adhesion plugs to the glass tool.

4. Discussion

The results of the experiments show that cells manipulated by ultra slow tools with tip diameters smaller than cells are not destroyed irrespective of the tip geometry. During an ultra slow manipulation the velocity is adapted in relation to cytoskeleton and membrane reorganization processes (figure 8). Previous work on microinjection has shown that rapidly moved micro tools

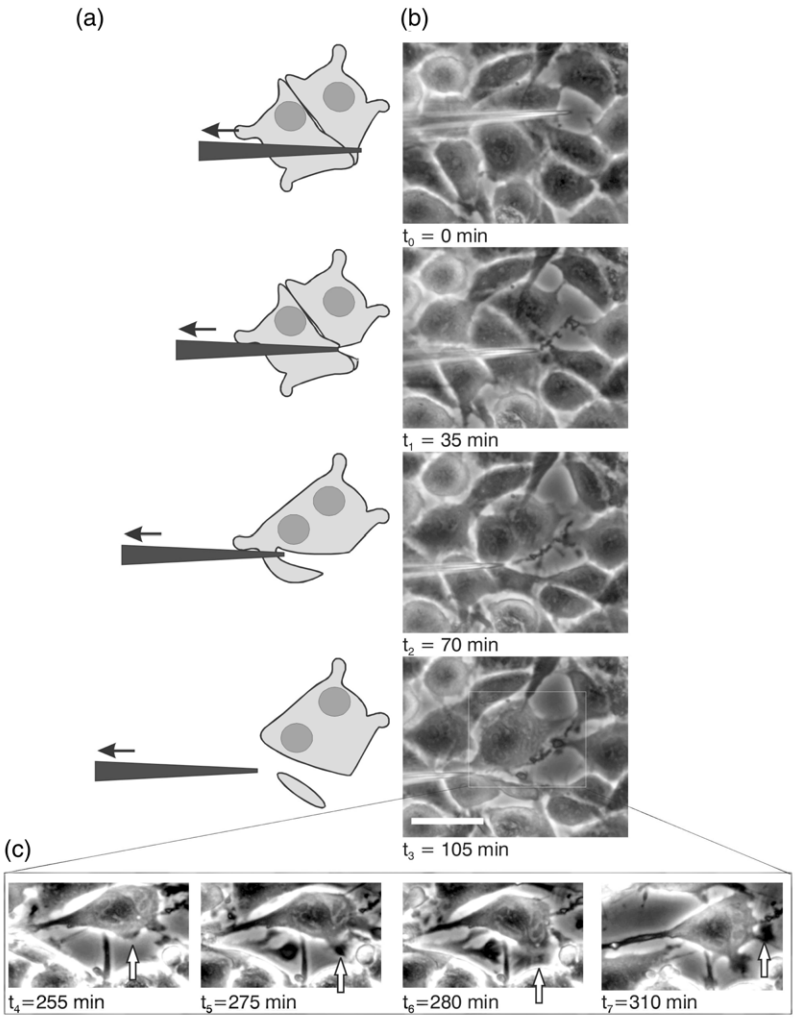

Figure 5. Fusion of two L-929 cells caused by a slowly moved thin glass tip (diameter of the glass tip 300 nm). (a) Schematic drawing of the procedure and cell behaviour. (b) Sequences of phase contrast images of the procedure. The images correspond to the schemes on the left side. Bar = 30 μm. (c) Sequence of phase contrast images of the fused cell. The arrows indicate the cytoskeletal activity.

can also be inserted in a cell and removed without destroying the cell [11]. However, in the case of fast microinjection the tip geometry is of importance [12]. In contrast to creep manipulation during a fast manipulation the velocity is not adapted to the molecular reorganization processes (figure 8). A future challenge is to provide data to enable a quantitative explanation of the effects of fast and slow manipulations on the rheology of cytoskeleton, of plasma membrane and on the molecular processes of the cells. Many advantages in microrheology and imaging have been used to quantify cell mechanics and to pinpoint the molecular substructures that might determine the mechanical properties of cells [13–17]. However, a theoretical model that relates molecular structures to viscoelasticity has not been fully developed for the polymers of the cytoskeleton [18].

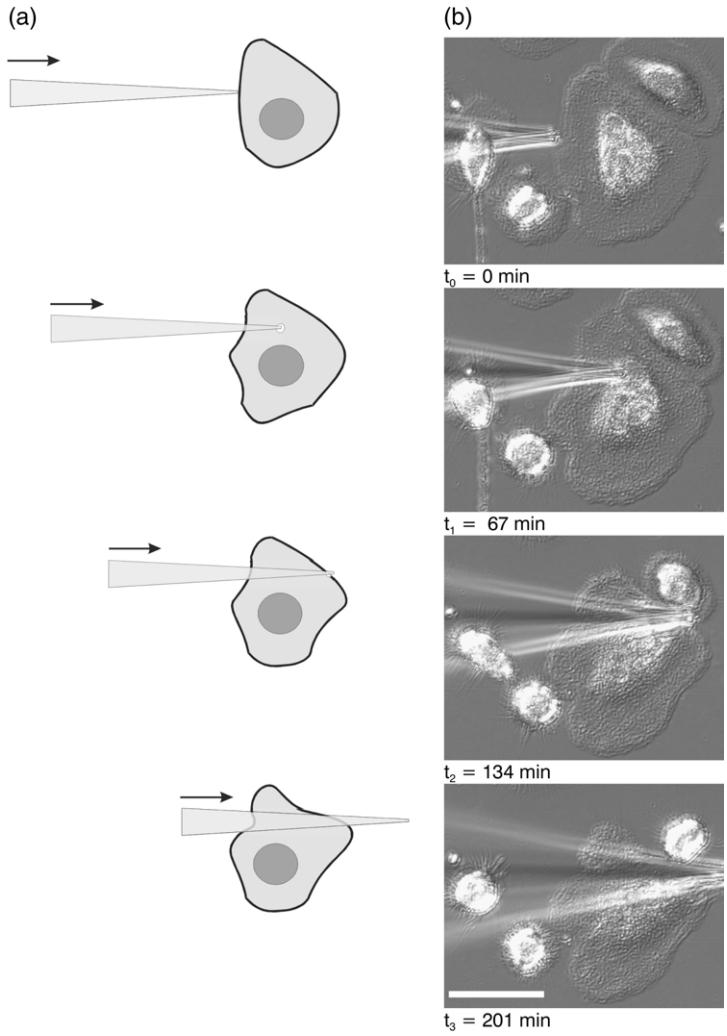

Figure 6. Movement of a thin glass tip (diameter 300 nm) through a macrophage. (a) Schematic drawing of the procedure. (b) Sequence of phase contrast images (Bar = 30 μm).

In the last groups of experiments we have demonstrated the movement of adherent cells to a certain position. To our knowledge it is not possible to move adherent cell by forces generated by optical tweezers, dielectrophoresis or ultrasound traps, since the adhesion forces of cells are in the range from some nN to several μN [15, 19]. Optical tweezers, dielectrophoresis or ultrasound traps for gentle cell manipulation generate forces on cells in the range of 1–100 pN [20]. Magnetic tweezers allow one to apply forces in the nN range to cells [21]. Therefore magnetic beats are coupled to various integins located in the cell membrane by coating the beats with fibronectin or invasin [17, 21].

The possibility of separating a sample of cytoplasm enveloped by plasma membrane without damaging the cell or to cut a part of a cell allows a variety of biochemical, genetic, immunological and other characterizations of individual cells. Cells can be characterized without contamination by cutting and separating parts which can then be analysed by

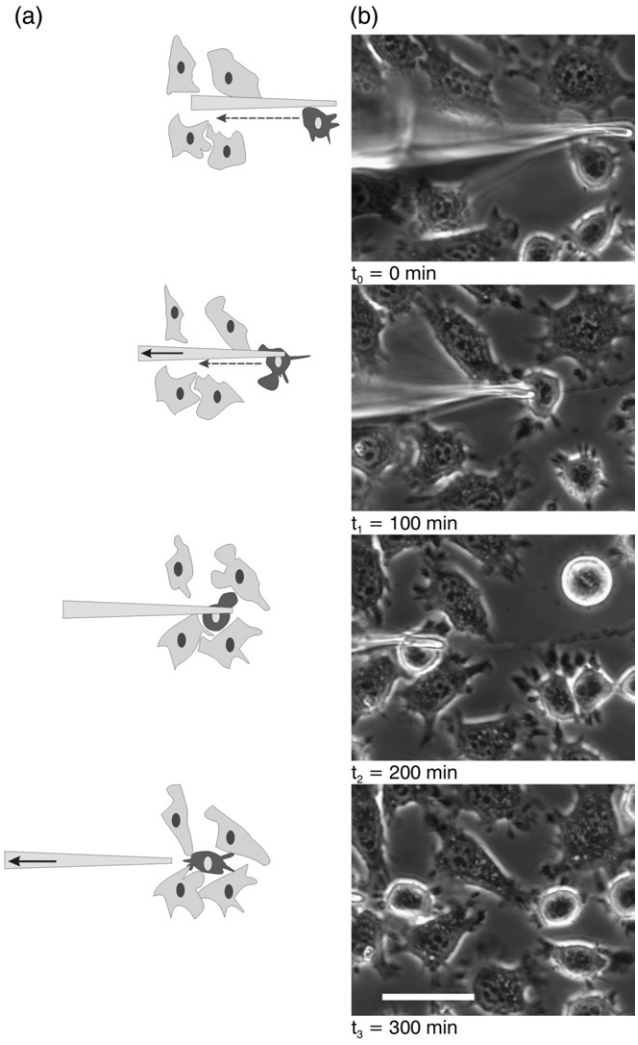

Figure 7. Grouping of adherent L-929 cells by using an ultra slowly moved bent tip. (a) Schematic drawing of the procedure. (b) Sequences of phase contrast images (Bar = 30 μm).

destructive or non-destructive methods. As shown previously [22], selected cells can be removed from cell monolayers without trypsinization and mechanical stress. The demonstrated cell manipulation could be important for cell handling, especially for cloning in the case of stem cell applications in medicine. Therefore, we tested our procedures and equipment not only on fibroblast, but also on macrophages and adult stem cells. Active migration of small separated cell parts permits fairly easy isolation (and transformation as recently described [6]). Polymerase chain reaction (PCR) analysis or specific immunohistochemical characterization is only a question of sensitivity and a miniaturized assay system. The experiments described here were done repeatedly and are reproducible. Some recent reports suggest that cell fusion may explain some important effects of stem cell biology [22, 23]. The controlled fusion of adherently growing cells under defined *in vitro* conditions should support the investigation of these processes under detailed microscopic observation. The manipulation technique

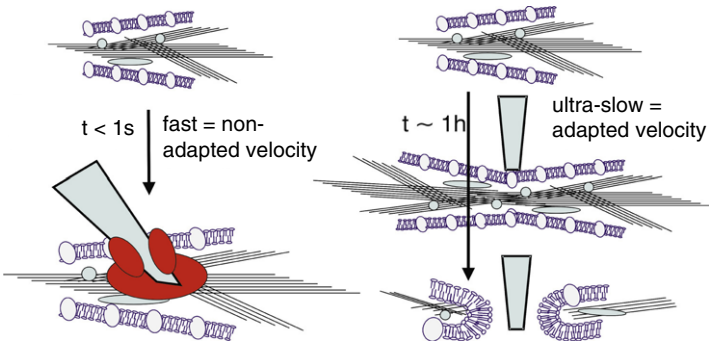

Figure 8. Comparison between cell manipulation by fast and ultra slow instruments. Schematic drawings to illustrate the interaction of a rapidly moved micro tool (left) and an ultra slowly moved micro tool. During an ultra slow manipulation the velocity is adapted in relation to cytoskeleton and membrane reorganization processes. In contrast, during a fast manipulation the velocity is not adapted.

presented here allows simple cell biopsy and fusion in an automated and physiological manner and supports the investigation of cell–cell compatibility, stem cell differentiation and cell interactions. Some commercially available micromanipulation systems can be modified to realize these manipulation techniques allowing their rapid introduction into biotechnology and basic research. To be as slow as cells are is a physiological way of making cell manipulation individual, automated and gentle.

5. Conclusion

The manipulation of cells with tools moved so slowly that the cytoskeleton and membrane system of the target cell can rearrange in a natural way and corresponding to the active migration velocity of cells has been verified. This allows the cutting of part from a cell without destroying the cell, the controlled fusion of adherently growing cells, the movement of instruments inside a cell without damage and the controlled migration of cells on a surface. The applied instrument velocities are lower by a factor of 500–1000 than the common velocities used in biology and medicine. The possibility of separating a sample of cytoplasm enveloped by plasma membrane without damaging the cell allows a variety of biochemical, genetic, immunological and other characterizations of individual cells.

Acknowledgments

We are grateful to Stephen G Shirley for a critical reading of the manuscript and for helpful discussions. We thank Erwin Gorjup, Pia Bartholomä and Sybille Kofa for expert technical assistance.

References

[1] Lewis R 1990 *Photon. Spectra* (July) 74–8
[2] Schutze K and Clement-Sengewald A 1994 *Nature* **368** 667–9
[3] Greulichand K O and Pilarczyk G 1998 *Cell Mol. Biol. (Noisy-le-grand)* **44** 701–10
[4] Zimmerman U, Friedrich U, Mussauer H, Gessner P, Hämel K and Sukhorukov V L 2000 *Plasma Sci.* **28** 72–82
[5] Schnelle T, Muller T, Gradl G, Shirley S G and Fuhr G 2000 *Electrophoresis* **21** 66–73

[6] Thielecke H, Impidjati, Zimmermann H and Fuhr G R 2005 *Microsyst. Technol.* **11** 1230–41
[7] Zimmermann H, Richter E, Reichle C, Westphal I, Geggier P, Rehn U, Rogaschewski S, Bleiss W and Fuhr G R 2001 *Appl. Phys.* A **73** 11–26
[8] Kruse C, Birth M, Rohwedel J, Assmuth K, Goepel A and Wedel T 2004 *Appl. Phys.* A **79** 1617–24
[9] von Briesen H, Andreesen R, Esser R, Brugger W, Meichsner C, Becker K and Rubsamen-Waigmann H 1990 *Res. Virol.* **141** 225–31
[10] Kreutz M, Eisert V, Rubsamen-Waigmann H, Andreesen R and von Briesen H 1998 *AIDS Res. Human Retroviruses* **14** 1581–8
[11] McElligott M A and Dice J F 1984 *Biosci. Rep.* **4** 451–66
[12] McAllister D V, Wang P M, Davis S P, Park J H, Canatella P J, Allen M G and Prausnitz M R 2003 *Proc. Natl Acad. Sci.* **100** 13755–60
[13] Mizuno T, Kagami O, Sakai T and Kawasaki K 1996 *Cell Motil. Cytoskeleton* **35** 289–97
[14] Guck J, Ananthakrishnan R, Mahmood H, Moon TJ, Cunningham C C and Kas J 2001 *Biophys. J.* **81** 767–84
[15] du Roure O, Saez A, Buguin A, Austin R H, Chavrier P, Silberzan P and Ladoux B 2005 *Proc. Natl Acad. Sci.* **102** 2390–5
[16] Knight A E, Veigel C, Chambers C and Molloy J E 2001 *Prog. Biophys. Mol. Biol.* **77** 45–72
[17] Feneberg W, Aepfelbacher M and Sackmann E 2004 *Biophys. J.* **87** 1338–50
[18] Janmey P A and Weitz D A 2004 *Trends Biochem. Sci.* **29** 364–70
[19] Benoit M and Gaub H E 2002 *Cells Tissues Organs* **172** 174–89
[20] Fuhr G and Reichle C 2000 *Trends Anal. Chem.* **19** 402–9
[21] Bausch A R, Ziemann F, Boulbitch A A, Jacobson K and Sackmann E 1998 *Biophys. J.* **75** 2038–49
[22] Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz D M, Nakano Y, Meyer E M, Morel L, Petersen B E and Scott E W 2002 *Nature* **416** 542–5
[23] Ying Q Y, Nichols J, Evans E P and Smith A G 2002 *Nature* **416** 545–8