HIGH FREQUENCY ELECTRIC FIELDS FOR TRAPPING OF VIRUSES

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SUMMARY

Combining dielectrophoretic and hydrodynamic forces in micro electrode structures allows enrichment and stable trapping of viruses in aqueous solutions. Fluorescently labelled Influenza and Sendai viruses were collected from solutions of 2*10^5 - 2*10^8 viruses/µl within a few seconds. In the central part of the trap a virus aggregate of about 2-9 µm in diameter was formed. This corresponds to a local enrichment of viruses up to a factor of about 1400.

INTRODUCTION

Under appropriate conditions, alternating (a.c.) electric fields give dielectric polarisation forces which repel particles of lower effective permittivity than the surrounding medium from electrodes (Pohl, 1978). Planar, quadrupole microelectrode-arrangements combine these forces with sedimentation and hydrodynamic forces to entrap suspended particles in "field funnels" or traps. Three-dimensional, octupole electrode-arrangements allow the creation of electric field cages, which entrap objects by polarisation forces only. For suspended submicron particles (such as viruses), Brownian motion becomes significant. Polarisation forces scale with the third power of the particle radius whereas thermal motions vary inversely with it. If these were the only considerations, particles with radii less than 500 nm could not be trapped in water filled field cages (Pohl, 1978). However, miniaturised electrode- assemblies allow the application of larger and more inhomogeneous electric fields (due to increase in breakdown strength of aqueous solutions), both increase the polarisation forces. Recently, it was shown that latex beads up to a diameter of 14 nm can be concentrated (Müller et al., 1995). This should also opens up new perspectives for handling of bacteria, viruses and macromolecules.
MATERIALS AND METHODS

Microstructures: We have used electrode assemblies produced on glass wafers by optical lithography with dimensions typically between 5 and 25 μm (Fuhr et al., 1995). The planar electrodes were wetted with a 10 μl droplet of particle suspension and covered by a glass plate approximately 20 μm above the electrode surface. Closed field cages require a three-dimensional arrangement of electrodes. To achieve this, two planar electrode structures were mounted face to face, several micrometers apart (Fig. 1). The space between them is filled with a virus suspension, either directly or by structured micro-channel systems. The devices can be cleaned in an ultrasonic bath, cold or hot sterilised, and rinsed with alcohol or other liquids.

Field generation: a.c. square wave pulses were applied using a Hewlett Packard generator HP-8116A. The frequencies were between 100 kHz and 50 MHz and amplitudes between 1 and 28 V

Viruses: Influenza virus (strain A/Japan and PR 8/34) and Sendai (strain Z) (1mg protein/ml) were fluorescently labelled with Octadecylrhodamine B chloride (Molecular Probes, USA) of 10 μM at room temperature for 30 min in the dark, centrifuged, washed and resuspended in ice-cold phosphate-buffered 150 mM NaCl, then transferred to phosphate-buffered 300 mOsm sorbitol using a Sephadex G-75 column. The final conductivity was about 74 mS/m and the final concentration was 0.2-1 mg protein/ml (1 mg protein/ml ≥ 2.5 10^12 viruses/ml). Fusion activity of labelled viruses was proved by fluorescence dequenching assay (Hoekstra et al.,1984).

The virus behaviour and the evolution of the fluorescence signal was recorded by a microscope-video system (Leica Metallux 3, LD 50 objective with a CCD Micro Camera CS 3130 in shutter mode or with an Confocal Laser Scanning Microscope, Leica). The video processing was done with the software of the CLSM and a video printer. The whole experimental set up is shown in Fig. 1.

Fig. 1: Experimental set up
Shown are a photo of a structure (left), a schematic view of one of the four cages, marked by arrows (middle) and the driving conditions (right). Top and bottom electrode planes are separated by an channel forming spacer. Bar : 100 μm.