A NOVEL MOBILE MONITORING SYSTEM FOR FAST AND AUTOMATED BACTERIA DETECTION IN WATER

Christoph Heller, Ulrich Reidt, Andreas Helwig, Florian Klettner, Gerhard Müller, Alois Friedberger
EADS Innovation Works, IW-SI – Sensors, Electronics & Systems Integration, 81663 Munich, Germany

Leonhard Meixner, Karl Neumeier
FhG-IZM Fraunhofer-Institut für Zuverlässigkeit und Mikrointegration, Hansastr. 27d, 80686 Munich, Germany

Petra Lindner, Ramona Molz and Hans Wolf
University of Regensburg, 93053 Regensburg, Germany

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Abstract: Standard detection methods for viable bacteria in potable water are time consuming due to a required cultivation step. Fast and automated detection of water borne microorganisms with high sensitivity and selectivity is still a challenge. We report on a novel biosensor using micromechanical filters with nano sized pores to capture and enrich bacteria on the filter surface and subsequent detection using fluorescent probes. The whole process is fully automated by integrating the sieves into a fluidic system together with a high performance fluorescence detector. The results show the effective retention of bacteria on the filter surface, which are then accessible for different staining procedures. As an example, we use special fluorescent dyes that bind to or intercalate in the DNA molecules of the bacteria. After detection, the microfilters undergo cleaning and conditioning steps to be ready for the next measurement.

1 INTRODUCTION

Under normal circumstances, our drinking water is of very high quality. According to the current directive of the European Community, no viable *E. coli* may be present in a 250 mL sample of potable water. To ensure this high quality, water works and water suppliers are obliged to test the water quality at regular intervals.

For these tests, classical methods with cultivation on special growth media are still being used for routine applications. The water samples are plated on culturing plates and incubated at various temperatures. Depending on the type of bacteria, this cultivation step can take up to several days. After incubation, the number of viable bacteria is determined by visually counting the bacterial colonies (Brenner, 1996; Gracias, 2004). Obviously, this procedure is very tedious and time consuming and requires skilled and experienced laboratory personnel. The fact that a contamination with some bacterial species (such as *Legionella*) will only be detected after several days may lead to the outbreak of epidemics. This can be a potential danger to people with a weak immune system.

In the rare event of bacterial growth in parts of a water supply network, all pipes, valves, pumps, etc., have to be thoroughly cleaned and re-examined before they can be approved for further use. The time consuming procedure of classical bacterial testing will therefore lead to rather long down times of a water supply system. The same is true for the supply of clean production water in pharmaceutical industry and for mobile water supply systems (e.g. in ships).

To avoid such problems, a system is needed that can continuously and automatically sample water and test it quickly for the presence or absence of bacteria or other pathogens.

Here, we describe such a system that has been developed in our group. At the core of this device, there is a micromechanical filter (sieve) having a large number of pores (about 10^7) with a diameter of 0,45 µm (Figure 1). When passing water through such filters, any bacteria present in the sample will

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be effectively captured and retained on the filter surface. Once on the filter, they are easily accessible to any kind of specific or non-specific staining method (e.g. with DNA intercalating dyes, enzyme substrates, membrane stains, labelled antibodies or hybridization probes). Fluorescent labels are used in our monitoring equipment.

Micromechanical filters are highly discriminative and show a much better filtration performance (higher filtration rate) than conventional filters of the same size. Their absolutely flat surface does not allow any particle to penetrate deeper into the filter's structure. Any bacteria retained by the filter will be present at the surface, allowing easy detection and washing off afterwards. Some work has been reported on using microfilters which successfully separate particles by means of filtration (Hsiai, 2002; Xing, 1999). These filters have pore diameters of 6-12 µm. Ogura et al. reported the separation of deformed red blood cells using microfilters with larger pore size (Ogura, 1991a; b). Since the average size of bacteria, for example *E. coli*, is approximately 1µm, theses filters are not suitable for the discriminative enrichment of bacteria.

In this paper we describe the successful capture, labelling and detection of *E. coli* on the surface of micromechanical sieves. We have integrated the MEMS filters into a microfluidic system, allowing fully automated water analysis with this biosensor system.

2 MATERIALS AND METHODS

2.1 Capture and Enrichment of Bacteria

The micromechanical filters are composed of a Si_3N_4 membrane (5 x 5 mm, approx. 1 µm thick) supported by a silicon frame. The membrane is perforated, each pore with a diameter of 450 nm (Figure 1). In contrast to tissue or membrane based filters, particles are not trapped inside a three dimensional filter structure but on the surface. Therefore, the particles are directly accessible. Previous experiments show that bacteria trapped on the surface can be easily removed after filtration (Reidt, in press). A further advantage of the micromechanical filters is based on the fact that all pores are simultaneously etched after a photolithographic patterning step. This ensures that all pores have exactly the same diameter and their spacing is completely homogenous (Figure 1).

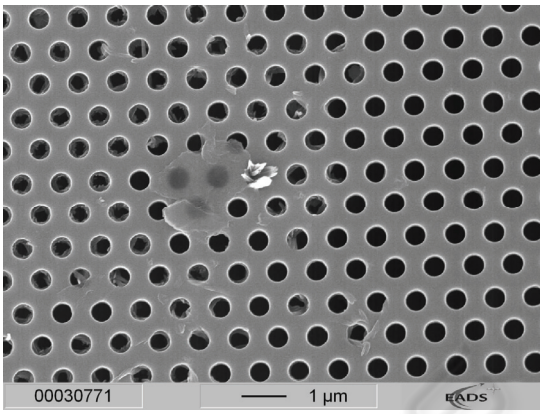

Figure 1: REM picture of a micromechanical filter surface with 0,45 µm diameter pore size (top view) after filtration of drinking water.

2.2 A Fully Automated System for Water Analysis

Using the micromechanical filters (sieves), we have developed a fully automated system for capturing, labelling and detecting bacteria in water. The sieves are housed in a microfluidic chamber with a glass window in very close distance above the filter surface. There is a water inlet close to the filter and one outlet in opposite position. Filter and glass window form a microfluidic channel, where liquid can be pumped from the inlet to the outlet passing in parallel to the filter surface (cross flow). A second outlet is positioned on the back side of the filter, allowing for flow through (dead end) filtration. Using a double selector valve, inlet and outlets can be activated to perform both types of flow. There is also the possibility to swap inlet and outlet for back flushing the filter (Figure 2). The maximum differential pressure over the microsieve is limited to 2 bar to avoid breaking the filter.

For detection, we use a standard setup with coaxial excitation and emission light paths. Light from a high power LED is collected and passed through an emission filter (485 +/-12 nm band pass). It is then reflected at 45° angle by a dichroic mirror (505 nm) and focussed onto the filter surface. The fluorescent light passes the mirror, is filtered through a 520 +/-15 nm band pass filter and focussed onto a photomultiplier tube (Figure 3). Device control and data acquisition is performed by LabView software on a laptop computer.

The precision pump (0 - 10 bar, 0.01 - 70 mL/min) was purchased from HNP (Parchim, Germany), whereas the selector valves were obtained from Valco-VICI (Schenkon, Switzerland). As detector we use a Perkin-Elmer MP962 photo

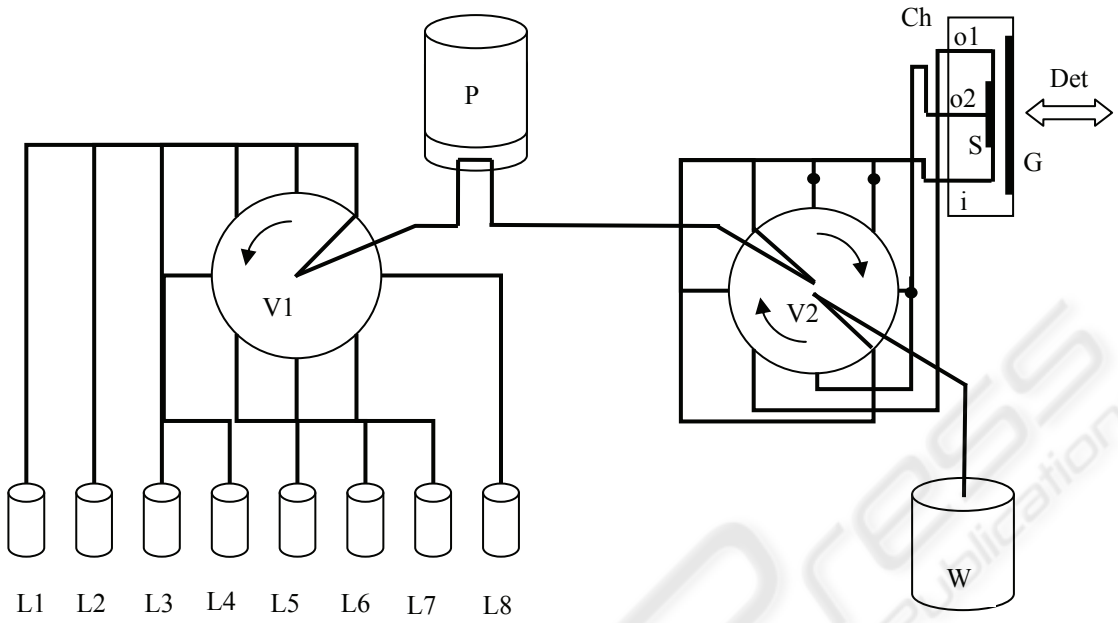

Figure 2: Scheme of the fluidic path in the automated detection system. L1 – L8: different liquids; V1: selector valve, V2: double selector valve, P: pump, W: waste, G: glass window, Ch: fluidic chamber, i: inlet, o1, o2: outlets, Det: detection system, S: micromechanical sieve.

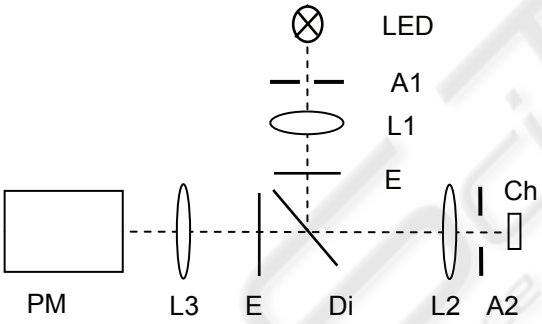

Figure 3: Scheme of the optical path in the automated detection system. LED: high power LED with lens, L1 – L3: lenses, A1, A2: apertures, Ex: excitation filter, Di: dichroic mirror, Em: emission filter, PM: photo multiplier, Ch: fluidic chamber.

multiplier; excitation is performed by a blue high power LED (Luxeon). Optical filters were from AHF Analysentechnik (Tübingen, Germany) and LabView software as well as a DAQ board were from National Instruments. Figure 4 shows a picture of the complete biosensor system which is small enough to be used as a mobile system.

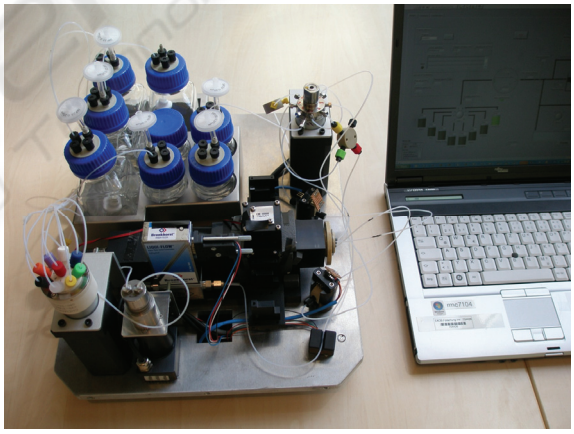

Figure 4: Picture of the automated monitoring system for bacteria in potable water.

2.3 Bacteria Labelling

For non specific labelling, 30 µL of a 100 fold dilution of SYBRGreen I (Invitrogen, Karlsruhe, Germany) was added to 1 mL bacterial suspension (about 10^8 cells/mL of heat inactivated *E. coli* O157:H7 from KPL, Gaithersburg, USA) and incubated for 15 min in the dark. From this stock solution serial dilutions were made in water and the exact numbers of bacteria were determined by

counting in an improved Neubauer counting chamber.

For immunodetection (specific detection) on the filter surface captured bacteria were overlaid with antibody solution specific for *E. coli* O157:H7 (polyclonal anti-*E. coli* O157:H7-Alexa 488 (ABL Advanced Biomedical, UK) 2.4 µg/ml) and incubated for 15 min. Non-bound antibodies were removed by an extensive washing step (100 ml, PBS) and bacteria recognized by the antibodies were identified by fluorescence microscopy (Reidt, in press).

3 RESULTS

3.1 Suitability of Micromechanical Filters for Capturing and Enrichment of Bacteria

In a first step, the suitability of micromechanical sieves for capturing bacteria was tested. 20 mL of various dilutions (10^2, 10^3 and 10^5 cells/mL) of a freshly prepared overnight culture of *E. coli* XL1-Blue were passed through the filter. After filtration, bacteria were removed from the filter surface by rinsing with 5 mL PBS (0.14 M NaCl, 0.01 M KCl, 0.008 M Na_2HPO_4, 0.0015 M KH_2PO_4, pH 7.4). In each case 100 µL of rinsing solution and filtrate (flow through) were plated on LB agar (10 g tryptone, 5 g yeast extract, 5 g NaCl to 1 liter H_2O) and incubated overnight at 37°C. Cell counts obtained by plating initially applied bacterial solutions (original solution) and from rinsing solutions correlate very well, indicating that bacteria can easily and efficiently be removed from the filter.

A major finding of all filter experiments was that in no case any bacteria could be detected in the 100 µL samples from the flow-through. The impermeability of the filters for bacteria was further confirmed by examination of the complete filtrate solution for the presence of bacteria. This demonstrates a highly efficient and reliable filtering process using the inorganic microsieves (Reidt, in press).

3.2 Bacteria Detection

For unspecific detection we use fluorescent dyes that bind to or intercalate in the DNA of the bacteria. Upon binding, the fluorescent signal of the dyes increases by a factor of 1.000-10.000.

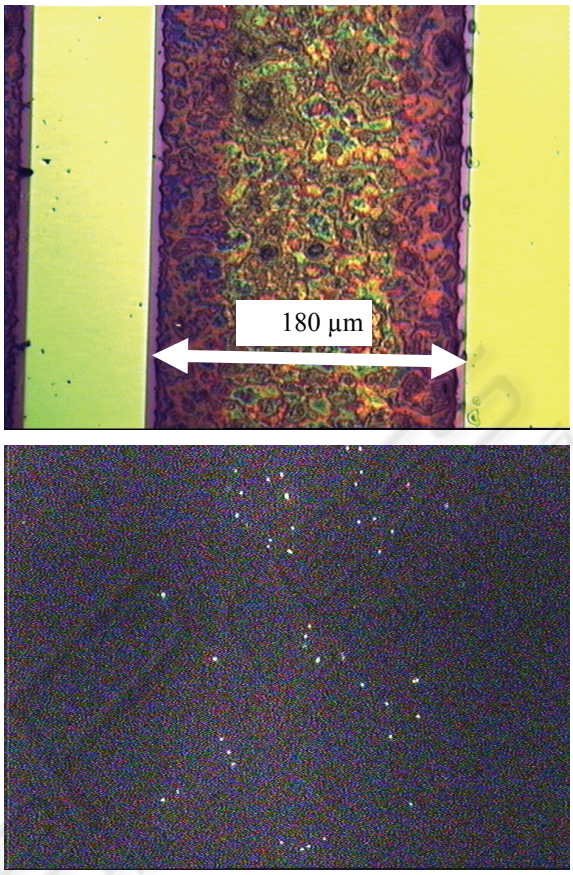

Figure 5: Upper: Microscopic image of part of the microsieve after filtering a water sample containing SYBRGreen I stained bacteria. The width of an active filter stripe is 180 µm. Lower: Same area, with fluorescence microscopy. The bright spots represent bacteria.

In our case inactivated *E. coli* O157:H7 are stained with SYBRGreen I. Water samples are spiked with different amounts of fluorescently labelled bacteria and analyzed in the monitoring system. Figure 5 shows a microscopic image of the filter after analysis and the corresponding fluorescent image visualizing the bacteria. So far, a limit of detection of 40.000 cells on a single microfilter could be achieved (Figure 6). The detection limit can be significantly improved as it is currently limited by the optical detection system not yet being optimized.

With the system, it is also possible to first pass a water sample (with non labelled bacteria) through the microsieve and then stain the cells on the filter surface.

Obviously, it should be possible to perform other specific or non specific labelling procedures. For example, we have also tested the immunodetection

of non-pathogenic *E. coli* O157:H7 on micromechanical filters. It could be demonstrated that *E. coli* serotype O157:H7 could be detected specifically by the antibodies while neither the micromechanical filter surface nor the negative control (*E. coli* DH5α) interacted with the antibodies (Reidt, in press).

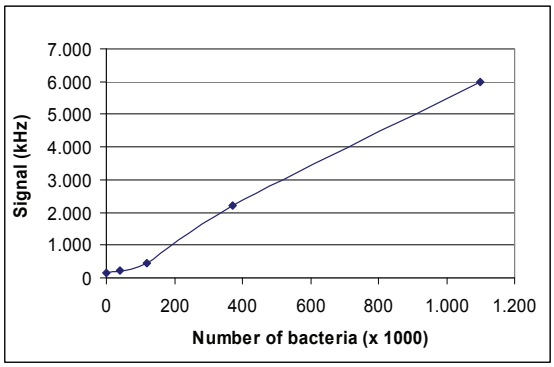

Figure 6: Detection of fluorescently labelled bacteria with a fully automated detection system.

Depending on the source, there may be potable water available in some regions with a quite high density of particles. This could cause problems in an automated water analysis system. One concern could be a background fluorescence signal originating from particles. Although the optical system is not optimized yet, we could not observe a significant signal from particles.

Another issue might be the blocking of the filters by the dirt. In fact, water flow rate of tap water through the sieves is reduced in comparison to pure water. However, a reasonable flow rate is still sustained (Figure 7). If the system should be used for water with a high density of particles, the microfilter area can simply be increased (currently it is 5 x 5 mm).

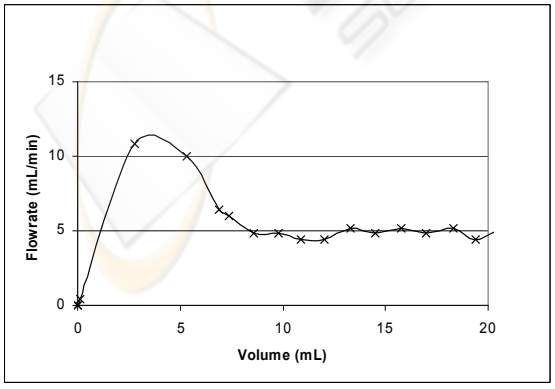

Figure 7: Flow rate in mL/min of tap water through a microsieve (microfilter) with size 5 x 5 mm.

4 DISCUSSION

We have shown that micromechanical filters can retain bacteria from water with high efficiency and reliability. Also, bacteria can directly be detected on the surface of the micromechanical filters using fluorescently labelled antibodies or DNA binding dyes. The microfilter platform is very flexible, i.e. other specific or non specific labelling procedures can be applied such as membrane probes, enzyme substrates, nucleic acid hybridization probes or PCR.

Furthermore, the micromechanical filters have been integrated into a fully automated fluid delivery and detection system. Micromechanical filters with appropriate pore sizes could also be an effective tool for recovering other pathogens such as viruses and protozoa.

During the last years, a few bacteria detection methods have been developed as alternatives to cultivation techniques, mainly based on flow cytometry (e.g., Sakamato, 2005) or solid phase cytometry (e.g., Aurell, 2004; Lisle, 2004). Flow cytometry is fast, but is only capable of analyzing a volume of a few microlitres per minute and thus requires preconcentration of the drinking water sample. In solid phase cytometry, the water is filtered through a porous membrane which has then to be transferred to a laser scanning device. Total analysis time is about 4 hours.

In both cases, a number of manual steps is required, making automation difficult, whereas our method offers the possibility of fully automated and rapid analysis of potable water samples.

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