Fast control of the effectiveness of UV disinfection with polyaniline nanobiodetector.

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ABSTRACT

New technique, based on a polyaniline continuous flow nanobiodetector (CFNBD), has been applied to monitor the progress of the UV light sterilization process against microorganisms (bacteria and yeast cells). CFNBD is able to measure the population of living cells and this feature is crucial for many applications, including fast control of the effectiveness of sterilization processes. The new analytical device of a high sensitivity and the very short response time can be applied to monitor instantly the population of microorganisms in the washing rinses solution, instead of a time consuming traditional procedure. In particular, the effectiveness of disinfection of spacecraft assembly facilities can easy be controlled (also in an on-line, fully automatic regime) and the time consuming traditional procedure can significantly be improved.

KEYWORDS:

Disinfection monitoring, UV disinfection, Polyaniline, Nanodetector, Biosensor, Flow analysis, Nanobiodetector.

1. Introduction

Recent great advance in nanotechnology have led to the development of new types of efficient and inexpensive sensors and detectors. Those novel devices are being designed and fabricated as a new class of analytical equipment working in the nano- or/and micro-scale (Vaseashtaa and Dimova-Malinovska, 2005).

A nanoscale sensing devices are ideal for various applications, including chemical sensing (Toal and Trogler, 2006), *in vivo* medical analysis (Shin and Schoenfisch, 2006; Blazej et al., 2006) and biological testing (Langer at al., 2007; Langer and Langer, 2005; Radke and Alocilja, 2004; Vo-Dinh et al., 2000a; Kasili et al., 2002; Cullum et al., 2000; Vo-Dinh and Cullum, 2000; Vo-Dinh et al., 2000b).

In some cases, a simple and basic, but fast biological analysis is much more useful and important than complicated, time consuming and expensive accurate measurements with traditional methods. For example, microbiological tests for the efficiency of sterilization (including UV light disinfection (Blatchley et al., 2001; Blatchley et al., 2005; Pennell, 2005) of spacecraft assembly facilities, such as those used by NASA at the California Institute of Technology's Jet Propulsion Laboratory (JPL), the Johnson Space Center, and the Kennedy Space Center, do not require – in fact – full identification of all microbial species. Up to date, NASA uses its 25-year-old standard assay (Jet Propulsion Laboratory, 1980) for enumeration of spores and heterotrophic microbial populations. The assay is based on viable counting techniques, such as washing of surfaces with a sterile phosphate-buffered rinse solution with mild sonication, after which the rinse solution is aseptically analyzed for numbers of microbes by standard pour plate techniques using media such as trypticase soy agar TSA (Crawford, 2005). This is a time consuming procedure.

Our method offers fast, instant monitoring of sterilization processes. The idea of a polyaniline nanobiodetector has been described elsewhere (Langer and Langer, 2005). The more advance device is a continuous flow nanobiodetector, CFNBD, designed recently and applied in this work.

2. The measuring system

Polyaniline CFNBD measuring system is a lab-on-a-chip-type device, which consists of a free standing polyaniline nanofibril network of 5000 μm^2 in size, formed in a narrow gap (about 1.5 μm) between two Au microelectrodes. The electron current flows through a relatively low number of nanofibrils of the average length of 1-1.5 μm and the thickness below 50 nm. To limit the ion current, the surface of Au electrodes was covered with a self-assembled monomolecular insulating layer, formed of benzylthiol. The system is working on the basis of a quantum effect associated with the reduced dimensionality of PANI nanofibrils and a modification of the local microenvironment by living cells strongly influencing the electrical properties of nanofibrils. This results in a high sensitivity and the very short response time (few seconds) of the nanobiodetector. In most of our experiments the data were collected within 150 seconds, but in fact, the readout time can be much shorter, e.g. 5-15 s (Langer and Langer, 2005).

Thus, the method is extremely fast comparing to 24h incubating process needed in standard pour plate techniques.

2. 1. Biological samples preparation and UV irradiation

For testing experiments, we have selected two types of microorganisms: yeast, *Saccharomyces cerevisiae* and bacteria, *Lactobacillus rhamnosus*. The biological samples (about 10^7 cells) have been suspended in 10 ml of bi-distilled water in a quartz reaction chamber (a cylinder of a diameter of 35 mm), and then irradiated separately for up to 60 minutes with the UV light (254 nm, generated by a resonance light source 9W) at a constant distance of 10 cm between the UV light source and the sample.

To prevent gravitational sedimentation the suspension of cells was stirred with a magnetic stirrer during the irradiation process.

2. 2. Measurements of the electrical response of CFNBD

Small samples of 125 μL of the suspension of cells were taken with a micro syringe and injected through septa into the cylindrical channel (the diameter of 2.7 mm) at a distance of 100 mm from the detecting device, CFNBD. The same was done with an automatic sampling system UNIPAN automatic dispenser type 336B (similar devices can also be applied), which is more appropriate in the case of on-line monitoring. Bi-distilled water was used as a medium to transport the samples analyzed through the system at a constant flow rate of 2 ml/min. The peristaltic pump type 315 ZALiMP (PL) equipped with a cushion tank (of 15 ml) was used.

The detecting area is very narrow, just of 1.5 μm, and oriented perpendicularly to the flow direction. This results in a good resolution.

The electrical response was registered as a change in the electrical conductivity of polyaniline nanofibrils. The electrical conductivity was measured with an accuracy of $\pm$0.01 μS using a conductivity meter CC-501 (Elmetron) operated in a computer-controlled mode. The data were collected automatically during 150 s after injection each of the samples and elaborated with the IBM PC station: calculation of the integral intensity of the signal vs. the base line (the integral signal intensity is much more accurate than the signal amplitude).

Samples were taken and analyzed with CFNBD after 0, 5, 10, 15, 20, 30, 45 and 60 minutes of the UV irradiation time (Fig. 1 a and b). At the same time the population of living cells in each of the samples was calculated directly with the standard Miles and Misry method (Singleton, 1999).

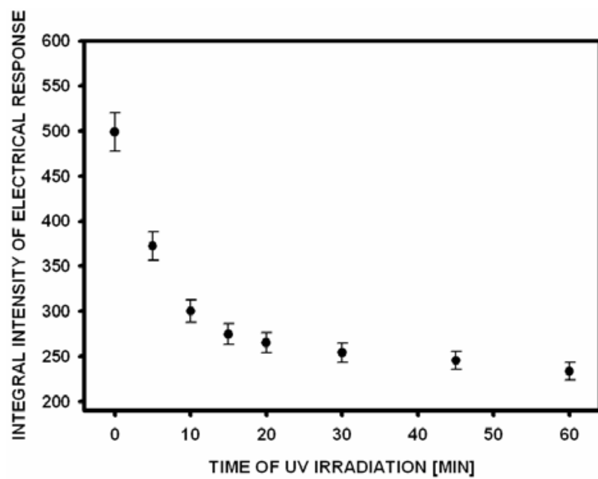

Fig. 1a

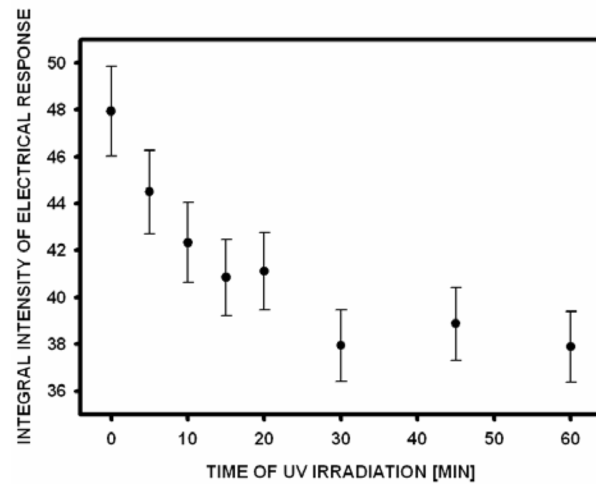

Fig. 1b

Fig. 1. A decrease in the integral electrical response of CFNBD with the time of UV irradiation for samples of *Saccharomyces crevisiae* (Fig 1a) and *Lactobacillus rhamnosu* (Fig 1b).

3. Monitoring of the UV light sterilization process against bacteria and yeast cells with CFNBD

The new technique, a polyaniline nanobiodetector working in a continuous flow system (CFNBD), has been applied to monitor the progress of the UV light sterilization process against bacteria and yeast cells. This is a demonstration experiment (a proof of concept) for more advanced applications, e.g. leading to improve NASA procedures. The paper presents a clear evidence for detection of living cells with CFNBD in the presence of killed cells.

Changes in the electrical response of CFNBD were registered for yeast cells and bacteria as a function of the UV irradiation time and presented in the form of a plot as an integral intensity of the electrical signal vs. time of irradiation (Fig. 1). To compare the results (the UV light biological effect) the population of living cells in each of the samples was calculated directly with the Miles and Misry method (Singleton, 1999).

In both cases, for yeast *Saccharomyces cerevisiae* (Fig 1a) and bacteria *Lactobacillus rhamnosus* (Fig 1b), a decrease in the population of living cells with the UV irradiation time is clearly observed and very well correlated with the CFNBD electrical response (Fig. 2). This is an exponential decay of living microorganisms (a decrease in the number of living cells).

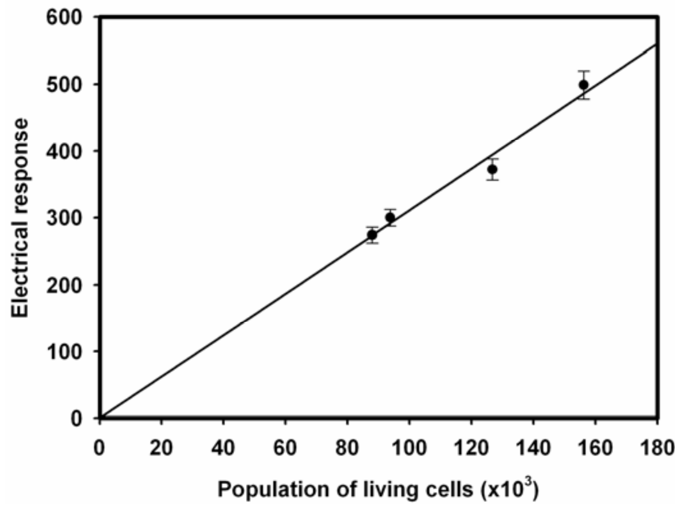

Fig. 2. Correlation of the electrical response of CFNBD and the population of living cells (*Saccharomyces cerevisiae*) in a sample analyzed (125μL) for first 15 minutes of UV irradiation.

The disinfection effect of UV on a single microorganism population is expressed as follows (VanOsdell and Foarde, 2002; Phillips Lighting Division, 1992):

$$N_t/N_0 = \exp(-t/\tau) = \exp(-k\, E_{eff} \cdot t) \qquad \text{(Eq. 1)}$$

where N_0 is the number of microorganisms at the start, N_t is the number of microorganisms after any time t, N_t/N_0 is the fraction of microorganisms surviving ($1-N_t/N_0$ is the fractional kill after time t), τ is the decay time (which depends on k and E_{eff}), k is a microorganism-dependent rate constant, $cm^2/\mu W \cdot s$, and E_{eff} is the effective irradiance received by the microorganism, $\mu W/cm^2$.

The applicability of Eq. 1 is well established for a microorganism exposed to a constant and uniform UV irradiance, as in our experiments. In our case, the values of the decay time τ for yeast and bacteria are comparable: 1.97 min and 2.06 min, respectively. This is reasonable, assuming a similar effective irradiance received by microorganisms E_{eff} (in both cases 254 nm UV is mainly absorbed by nucleic acid components) and comparable values of the rate constant k of the disinfection process (similar processes are responsible for killing the cells). After 20-30 min, the saturation is observed, as most of microorganisms are killed. This is a well-known UV irradiation effect.

Similar correlation was obtained with direct calculation of living cells in the samples analyzed using the Miles and Misry method. Thus, the electrical response of CFNBD is proportional to the population of living cells (Fig. 2) and the device can be applied in fast and convenient detecting systems to monitor microbiological contaminations.

As CFNBD is able to measure the population of living cells, which is crucial for many important applications, the effectiveness of disinfection of spacecraft assembly facilities can also easy be controlled and the traditional procedure can significantly be improved, including an on-line (fully automatic) system to monitor instantly the population of microorganisms in the washing rinse solution, instead of a time consuming traditional procedure (Fig.3).

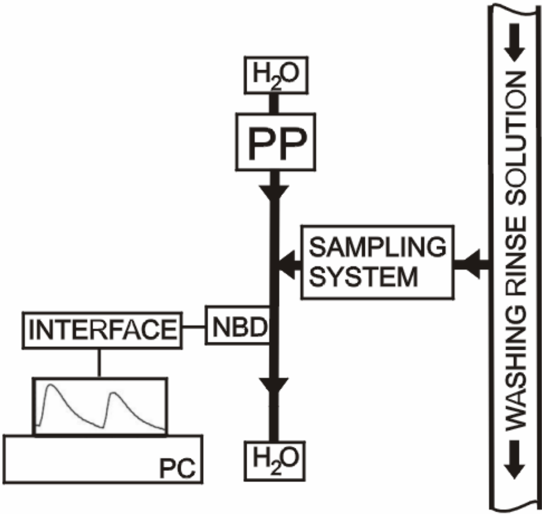

Fig. 3. A scheme of the on-line system to monitor the population of microorganisms in the washing rinse solution (NBD – polyaniline nanobiodetector, PC – computer, PP – peristaltic pump, H_2O - solvent (water) used as a medium to transport the samples analyzed).

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