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Tracking of the viability of *Stenotrophomonas maltophilia* bacteria population in polyvinylalcohol nanofiber webs by positron annihilation lifetime spectroscopy

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ABSTRACT

Polyvinylalcohol (PVA) fiber web containing embedded bacteria was prepared by electrospinning technique. From the point of the complex functionality of such potential delivery systems, it will be of impact how bacteria can survive in such artificial biotopes. The present study suggests a possible fast method for the tracking of the viability of the embedded bacteria based on the changes of the supramolecular structure of the polymeric delivery system caused by the metabolic product of the bacteria. Positron annihilation lifetime spectroscopy (PALS) was applied to track the free volume changes of the system in the course of storage. The PALS method sensitively detected the free volume changes, thus the viability of the bacteria in the polymeric fiber web.

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1. Introduction

Electrospinning is the cheapest and the most straightforward way to produce nanomaterials. It represents an attractive approach for polymer biomaterials processing, with the opportunity for control over morphology, porosity and composition using simple equipment (Poologasundarampillai et al., 2011). Because electrospinning is one of the few techniques to prepare long fibres of nano- to micro-metre diameter, great progress has been made in recent years. Superfine fibers could be produced by forcing a viscous polymer, composite, sol–gel solution or melt through a spinneret with an electric field to a droplet of the solution, most often at a metallic needle tip. The electric field draws this droplet into a structure called a Taylor cone. If the viscosity and surface tension of the solution are appropriately tuned, varicose break-up is avoided (if there is varicose break-up, then electrospray occurs) and a stable jet is formed (Marosi, 2010).

The incorporation of therapeutic compounds into the electrospun nanofibers has attracted a great deal of attention, because the resultant nanofiber webs have very strong efficacy of the drug due to their high surface area-to-volume ratio, and the composite electrospun nanofiber webs afforded the prospect of preparing useful polymer systems for controlled release of the activity (Tiwari et al., 2010). Poly(vinyl alcohol) (PVA) is a highly biocompatible water-soluble semicrystalline polymer the hydroxyl groups of which produce inter- and intra-molecular hydrogen bonding furthermore react readily with different cross-linking agents to form a gel (Song and Kim, 2004). These characteristics make PVA quite interesting for various pharmaceutical and biomedical applications (Hassan and Peppas, 2000). Although the number of papers published on electrospinning of PVA is quite large those of having pharmaceutical relevance are quite few (Agarwal et al., 2008). PVA nanofibers containing Donepezil HCl demonstrated their capability for achieving sudden release of drug if the formation of electrospun fibers is performed at suitable circumstances (Nagy et al., 2010). Green tea polyphenols (GTP), was encapsulated into biodegradable polymer nanofibers by an electrospinning process to impart controlled release function and a longer stability of chemical structure of GTP (Shao et al., 2011). The use of highly complex biological systems such as living bacteria or living cells is of great interest for the preparation of novel polymer systems with complex functionality. From the biological point of view, it will be of interest how bacteria can survive in such artificial biotopes (Gensheimer et al., 2011).

The purpose of the present study was to in situ track the supramolecular changes of electrospun polyvinylalcohol (PVA) nanofiber webs embedded *Stenotrophomonas maltophilia* bacteria by positron annihilation lifetime spectroscopy (PALS). The PALS method enables the detection of the free volume changes caused by the metabolic product, the resulting carbon dioxide, of living

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micro-organisms. The latter is of impact from the point of non-invasive testing of the viability of the system.

2. Experimental methods

2.1. Sample preparation by electrospinning method

25%w/w polyvinyl alcohol (Mowiol® 4-88, Mw ~31,000) aqueous solution was applied for the preparation of nanofibers. *S. maltophilia*, a gram-negative, non-fermentative, flagellated, rod-shaped bacterium was selected to embed into the nanofibers. *S. maltophilia* of 0.7–1.8 × 0.4–0.7 μm size dimensions was applied for the preparation of bacterium suspension according to the Ph. Eur. A liter of drinking water was removed to sterile bottle for the preparation of bacteria suspension.

The filtration and multiplication of bacteria was carried out in clean rooms type B and A and the appropriate wearing protective clothing was used in the course of the process. The mixing of the PVA solution with the suspension containing bacteria was prepared in the same clean area. The average bacterium content of the prepared suspension was 500 CFU/ml (colony-forming units per milliliter). 100 μl from the bacteria suspension was added to 10 ml PVA solution to obtain a polymeric mixture which was filled into a sterile syringe connected to the active area of the electrospinning device. Sterile tube and needle was set to the closed-tech equipment, which was placed in a type B biological safety cabinet. The electrostatic field overcomes the surface tension of the drops and stretches them towards the field direction. Nanofibers could be formed, depending on the circumstances, as a result of this process. During the fiber forming process the material loses most of its solvent and the dry nanofibers are deposited on a collector. By the end of the procedure the collector is covered with a tissue of randomly oriented nanofibers which can be removed from the receptive surface. The electrostatic spinner used for the experiments was equipped with NT-35 High Voltage DC Supply (MA2000). The utilized electrical potential, attached to the spinneret, was in the range of 20–25 kV. A grounded aluminum plate covered with aluminum foil was used as collector. The distance of the spinneret and the collector was 15 cm. The mixture was dosed by SEP-10S Plus syringe pump (Aitecs).

2.2. Sample storage

The fibrous samples were stored in closed containers at 40 °C ±0.5 °C and 75% ± 5% relative humidity for 3 weeks.

2.3. Morphology examination with scanning electron microscopy (SEM)

Fibrous samples were fixed on the sample holder using double-adhesive tape, and gold coating (JEOL 1200) was applied. Examinations were performed and images were taken using a JEOL JSM-6380LA type of scanning electron microscope (SEM). The accelerating voltage was 15 kV while the working distance was 10 mm.

2.4. FTIR spectroscopy

The ATR-FTIR spectra of the nanofibrous scaffolds of polyvinylalcohol embedded living *S. maltophilia* bacteria and that of polyvinylalcohol powdered substance (Ph. Eur.) scanned over wavenumber range of 4000–300 cm^{-1} using Able Jasco FTIR 4200 type spectrometer. 100 scans were performed at a resolution of 4 cm^{-1}.

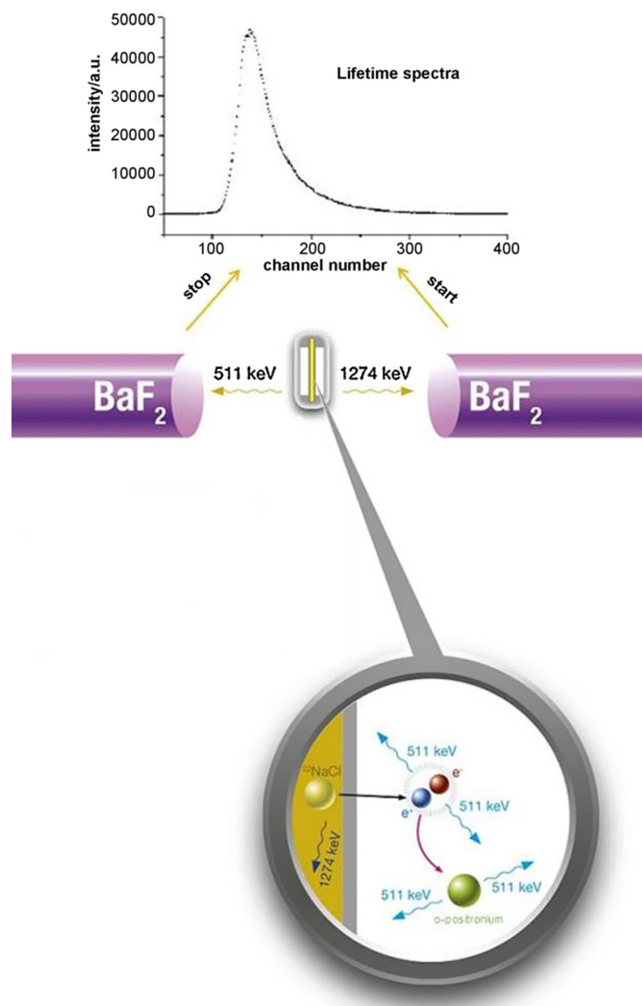

Fig. 1. The scheme of experimental setup of PALS measurements.

2.5. Positron annihilation lifetime measurements (PALS)

This method measures the size distribution of free volumes in polymers. For that, it applies positrons. The positron source applied for the measurements was made of carrier free 22NaCl of the activity of 105 Bq. The active sodium chloride was sealed between two very thin (2 μm) kapton foils. The source was then placed between two pieces of polymeric mixture treated identically before. Positron lifetime spectra were recorded by a conventional fast–fast coincidence system (MacKenzie, 1983). The system was constructed from standard ORTEC electronic units and the detectors from BaF_2 scintillator crystals and XP2020Q photomultipliers. The time resolution of the system was about 330 ps.

The spectra were first evaluated by the RESOLUTION computer code. Four lifetime components could be found in each case among which the two longest were identified as positronium lifetimes.

However, the discrete evaluation has revealed only minor changes. Thus, a variation of the MELT code (Shukla et al., 1993) was used to extract lifetime distributions from the spectra. This latter kind of evaluation gave a more detailed view of the changes, so, it was used to characterize the size distribution of free volume holes in the samples using *o*-Ps lifetimes. Fig. 1 illustrates the scheme of the experimental setup.

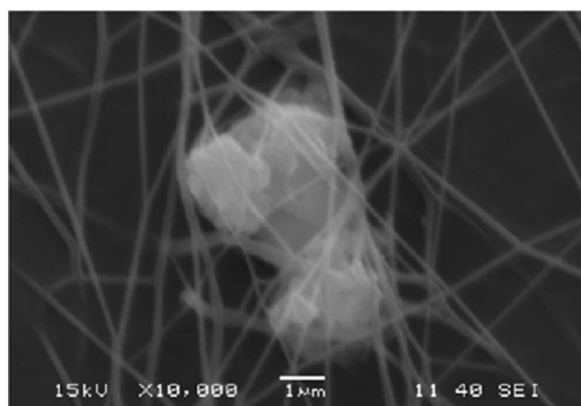

Fig. 2. Scanning electron microscopic photo of PVA nanofiber webs containing the embedded microorganism.

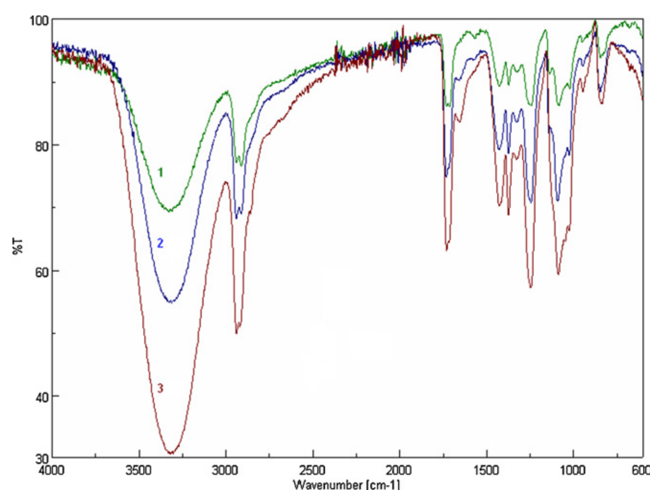

Fig. 3. ATR-FTIR spectra of powdered PVA and of nanofibrous scaffolds of PVA embedded living *Stenotrophomonas maltophilia*. Sample 1 – powdered PVA substance. Sample 2 – PVA nanofibers embedded bacteria without storage. Sample 3 – PVA nanofibers embedded bacteria after 1 week of storage.

3. Results and discussion

The SEM photo (Fig. 2) represents the morphology of the PVA electrospun fibers embedded microorganism. The embedded microorganism of flagellated rod shape refers to the *S. maltophilia*.

Fig. 3 illustrates the ATR-FTIR spectra of powdered PVA and of nanofibrous scaffolds of PVA embedded living *S. maltophilia* without storage and after a very short period of storage interval. The results indicate that the characteristic peaks of the pure powdered PVA substance were not changed in the presence of bacteria even after relatively short storage interval. It means that the bacteria did not change the molecular structure of the electrospun nanofibers.

Although the molecular structure remained intact, the supramolecular structure of the polymeric chains was significantly changed after 1 week storage independently of the presence of bacteria in the nanofiber web due to the swelling ability of the PVA. Fig. 4 indicates that after 1 week of storage, the *o*-Ps lifetime values of PVA nanofiber webs embedded *S. maltophilia* shifted to higher values, thus referring to higher free volume holes. The obtained phenomena could be explained with the metabolic product of bacteria which increased the free volume holes within the polymeric chains, similarly to the large quantities of carbon dioxide production from yeast. The metabolic product of the bacteria, the carbon dioxide could puff up the PVA nanofiber webs, thus changing the supramolecular structure, meanwhile the molecular structure of the polymer remained unchanged. After 2 week of storage, in contrast to the empty samples, the *o*-Ps lifetime values of sample containing bacteria did not change significantly, indicating that the bacteria survived the storage conditions. Microbiology test of the samples confirmed the obtained results. The volume expansion and later the collapse of the fiber web could be visually observed in both cases.

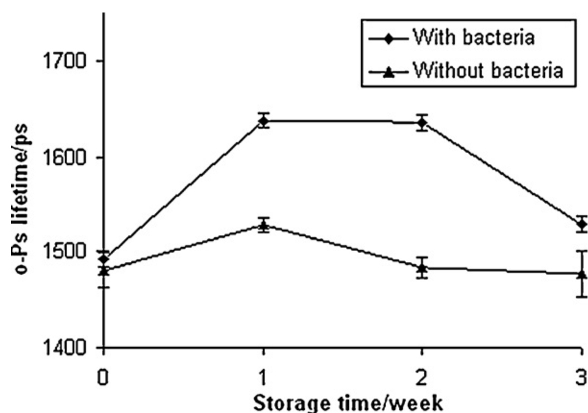

Fig. 4. Changes of the *o*-Ps lifetime values of u nanofibrous scaffolds of PVA embedded living *Stenotrophomonas maltophilia* as a function of storage time.

4. Conclusions

The PALS method enables non-destructive in situ investigations for bacteria viability in polymeric delivery systems based on the changes of the size-distribution of the polymeric free volume holes.

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