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A comparative study of two advanced spraying techniques for the deposition of biologically active enzyme coatings onto bone-substituting implants

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ABSTRACT

Surface modification of implant materials with biomolecule coatings is of high importance to improve implant fixation in bone tissue. In the current study, we present two techniques for the deposition of biologically active enzyme coatings onto implant materials. The well-established thin film ElectroSpray Deposition (ESD) technique was compared with the SAW-ED technique that combines high-frequency Surface Acoustic Wave atomization with Electrostatic Deposition. By immobilizing the enzyme alkaline phosphatase (ALP) onto implant surfaces, the influence of both SAW-ED and ESD deposition parameters on ALP deposition efficiency and ALP biological activity was investigated. ALP coatings with preserved enzyme activity were deposited by means of both the SAW-ED and ESD technique. The advantages of SAW-ED over ESD include the possibility to spray highly conductive protein solutions, and the 60-times faster deposition rate. Furthermore, significantly higher deposition efficiencies were observed for the SAW-ED technique compared to ESD. Generally, it was shown that protein inactivation is highly dependent on both droplet dehydration and the applied electrical field strength. The current study shows that SAW-ED is a versatile and flexible technique for the fabrication of functionally active biomolecule coatings.

1. Introduction

The biological performance of orthopedic and dental implants can be significantly improved by modifying a non-physiological metallic implant surface through the application of biologically active coatings. Physical adsorption and covalent attachment are methods widely used to immobilize biomolecules (such as extracellular matrix proteins, peptide sequences, bone growth factors and deoxyribonucleic acid) onto titanium (Ti) surfaces to enhance bone regeneration at the interface of implant devices [1–4]. However, by dipping Ti implants into a solution of proteins, biomolecules adsorb to the surface in an uncontrolled manner. Furthermore, surface loading is very low compared to methods such as covalent coupling. Covalent coupling, on the other hand, is generally a complex, multi-step process. To overcome these problems, one-step spray deposition techniques are of high interest for the fabrication of biomolecule coatings. In view of this, electrospray deposition (ESD) has already been shown to be very promising for the fabrication of both biomedical implant coatings and biosensor chips [5–7]. The ESD technique involves atomization of a protein-containing solution by applying a high voltage to a liquid surface, which then disperses into a spray of micron-sized, charged droplets (<10 μm; Fig. 1). The inherent small droplet size of this technique allows deposition of a thin dried biofilm onto the substrate surfaces without detrimental effects on the biological activity of biomolecules [8]. Further advantages of the ESD technique are (i) the very simple and cheap set-up, (ii) a high deposition efficiency, since the electric field directs charged droplets to the substrate, (iii) control over coating composition, and (iv) the possibility to tailor the morphology of the deposited coatings [7,9,10]. Although ESD is a versatile technique with many possible applications, a number of inherent problems related to this technique have limited the use of ESD to laboratory scale. The main drawback of electrospraying is that the process is restricted to diluted precursor solutions with low conductivity (<5 mS/cm) [11]. As a consequence, electrospray deposition rate is inherently low. To overcome these disadvantages of ESD, an advanced spraying method for the fabrication of functionally active protein chips was developed by Kim et al. that combines high-frequency surface acoustic wave atomization with electrostatic deposition (SAW-ED) [12]. A surface acoustic wave (SAW) is a Rayleigh wave propagating along the surface with an amplitude of tens of nanometers, generated by an

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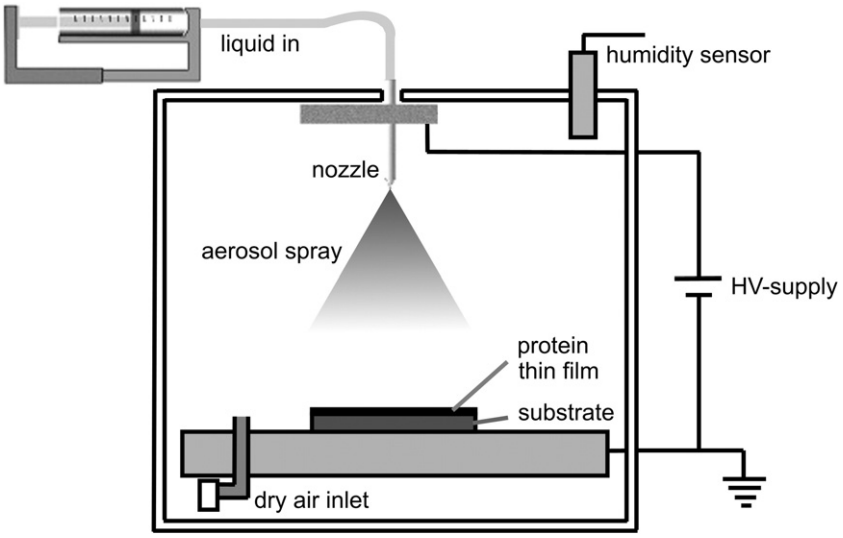

Fig. 1. Experimental set-up of the ESD technique.

interdigital transducer (IDT) installed on the surface of a piezoelectric substrate (Fig. 2) [13–17]. This IDT converts electrical signals into SAWs that strongly interact with small amounts of protein solution on the surface of the piezoelectric substrate. The propagating SAW radiates its energy into the liquid, causing the protein solution to vibrate, flow, and atomize. The liquid dynamic depends on the SAW amplitude, which can be controlled by the amplitude of the electrical input signal. Employing liquid surface vibrations of about 10 MHz, the SAW device atomizes independently of any externally applied potential difference. In combination with the electrospraying principle, the small droplets (<10 μm) atomized by the SAW device are directed towards the implant surface by applying a high voltage between the liquid and a grounded implant material (Fig. 3). Similar to ESD, the main advantage of this SAW-ED technique for application in the biomedical field is the fast protein dehydration process due to the micro-scale of the droplets, which reduces loss of protein biological activity [8]. In addition, the main improvement of the combined SAW-ED over ESD for biomolecule coating fabrication is the possibility to spray highly concentrated and conductive protein solutions, since droplets are generated by acoustic vibrations. Another feature of SAW-ED that is favorable to the ESD technique includes the fast deposition rate, since droplets are emitted from the entire protein solution surface on the substrate instead from a nozzle with a micron-sized diameter. As such, SAW-ED would allow deposition of practically every protein solution onto implant materials.

So far, the SAW-ED technique has been investigated by Kim et al. with respect to spot-like deposition of biomolecules for the fabrication of protein microarrays [12]. However, the potential of SAW-ED for biomedical coating applications, more specifically functionally active protein layers, is still unexplored. Therefore, the objective of this study was to investigate the feasibility of the SAW-ED technique to deposit biologically active protein coatings onto implant surfaces. Alkaline phosphatase (ALP) was chosen for this investigation, since the biological activity of this enzyme can be quantified easily using a simple biochemical assay. As such, the influence of SAW-ED deposition parameters on enzymatic activity of ALP could be assessed. Furthermore, ALP is of high interest for biomedical coatings to obtain early and strong implant fixation into native bone tissue, since ALP coatings are known to trigger early, enzymatically controlled stages of biomineralization [5]. In the current study, ALP coatings were deposited using the SAW-ED technique and compared to ALP depositions from the established ESD technique.

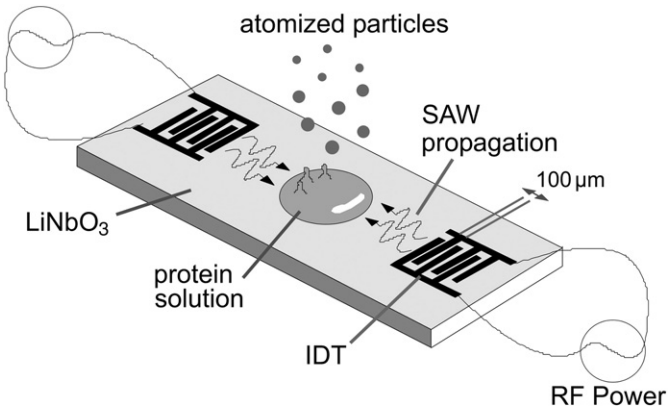

Fig. 2. Schematic illustration of the surface acoustic wave (SAW) atomizer.

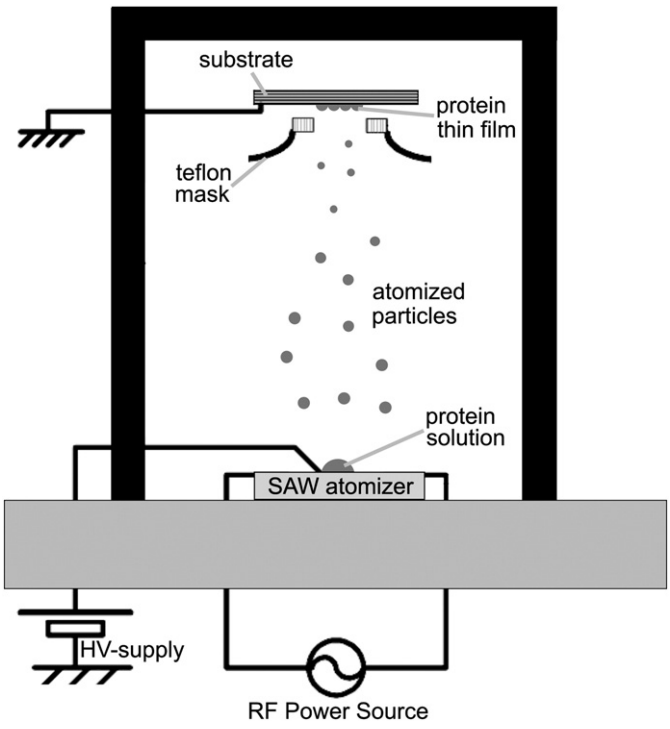

Fig. 3. Experimental set-up of the SAW-ED technique.