BIOMOLECULE COUPLING USING ALTERNATING CURRENT ELECTROPHORETIC DEPOSITION: PROTEIN, ENZYME, AND POLYSACCHARIDE COATINGS

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To control the host tissue response upon implantation and thus achieve a better clinical performance, the incorporation of bioactive molecules, such as proteins, enzymes or polysaccharides, at the implant surface by covalent bonding is an increasingly attractive strategy. Indeed, this approach allows better preservation of the stability of the molecules in physiological conditions. Dopamine (DA) self-polymerizes to polydopamine (PDA) in alkaline conditions with the accompanied oxidation of catechol groups to quinone and forms an intermediate layer with functional groups for covalently anchoring of bioactive molecules onto a wide variety of materials, amongst others titanium (Ti). In addition, to more rapidly attain a therapeutically active threshold concentration of these molecules at the surface, electrophoretic deposition (EPD) can be used to improve the immobilization yield. More specifically, in this study, we investigated the use of alternating current (AC) fields as this enables the fast processing of bioactive molecules from aqueous suspensions without compromising its biological activity[1]. First, the potential of AC-EPD to coat PDA pre-functionalized Ti surfaces was investigated using the model molecule bovine serum albumin (BSA). Thorough surface characterization, combining scanning electron and confocal microscopy, ellipsometry, contact angle, infrared spectroscopy and time-of-flight flight secondary ion mass spectrometry (ToF-SIMS) revealed a synergistic effect of the PDA coupling chemistry in combination with AC electrophoresis. Next, this AC-EPD immobilization protocol was transferred to a clinically relevant molecule, i.e. deoxyribonuclease (DNase) I, a DNA degrading enzyme targeting the extracellular DNA of bacterial biofilm matrices which allows to indirectly combat implant associated infections (IAIs). Surface characterization confirmed the increased efficacy of the AC-EPD process relative to traditional diffusion methodologies (i.e., simple dipping). This was further corroborated by enzymatic activity assays as well as in vitro biofilm experimentation using single species cultures of two different pathogenic species, Staphylococcus epidermidis and Pseudomonas aeruginosa[1]. To prove the broad applicability of the technique, AC-EPD of larger polysaccharide molecules was also considered. To this end, the deposition of water-soluble chitosan (CS) on Ti implant surfaces was investigated. It was shown that AC-EPD yielded the production of appreciably thick antimicrobial CS coatings, which remained effective against bacterial biofilm formation of a co-culture of the commensal strain Streptococcus gordonii and the periopathogenic strain Porphyromonas gingivalis.

Overall, the results show that AC-EPD is a versatile method, allowing the time-efficient grafting of a wide variety of biomolecules onto Ti surfaces (Figure 1). While focusing on the prevention of peri-implant infections, a proof-of-concept is given for several antimicrobial compounds. Yet, the technique can potentially be transferred to other sensitive bioactive molecules thereby targeting a wider range of biomedical applications.

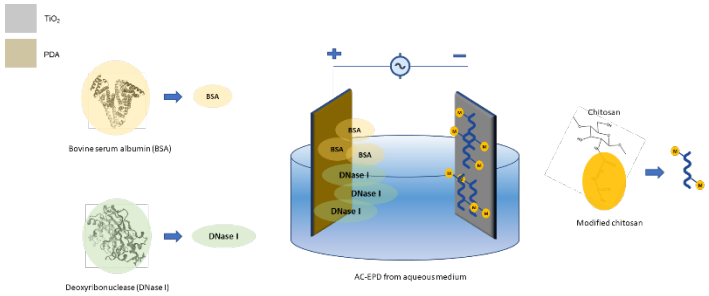

Figure 1 – Schematic representation of the alternating current electrophoretic deposition (AC-EPD) process for coupling a variety of biomolecules.

1. Aktan, M. K. *et al*. *Anal. Chim. Acta* 1218, 1–16 (2022).