Chapter 2

Analysis of the Bacterial Vesicles’ Enhanced Toxicological Threat Via Electron Microscopy

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ABSTRACT

This study shows the importance of electron microscopy in the analysis of the interaction of microorganisms (Staphylococcus aureus) with polymeric (polyurethane) dental prostheses. Starting from the biofilm formation and the biodestruction of the plastic material resulting in the production of polyurethane nanoparticles, the focus is on the bacterial secretion of membrane vesicles (in the range of 20-50 nm) loaded with plastic nanoparticles (from 2-3 to 10 nm) and on the toxicological threat that these delivery devices represent when interacting with host cells. The nanoparticles deliverance led by the bacterial infections dynamics opens new ways to the possibility of delivering drugs to selected cells.

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BACTERIAL BIOFILM, PLASTIC BIODESTRUCTION, AND NANOPARTICLES FORMATION

*Staphylococcus aureus* is a Gram-positive bacterium permanently present in the human body as a commensal, and often as a pathogen as well (Lowy, 1998). When polyurethane prosthetic devices come into contact with *S. aureus*, its ability to colonize medical implants, adhering to the polymeric surface and forming a biofilm, results in the development of a community with dynamics able to highly affect the material’s stability. The biofilm formation is a four-steps process (Figure 1) where the bacterial initial adhesion onto the polymer is followed by the formation of microcolonies, by the maturation phase with the appearance of the exopolysaccharide matrix and eventually by the detachment of nomadic cells (Didenko et al., 2012; Didenko et al., 2013; Curia et al., 2014; Ghannoum & O’Toole, 2004).

The action of a bacterial biofilm, characterized by high cell density and limitation of nutrients (Fux, Costerton, Stewart, & Stoodley, 2005), provokes damages on the prosthesis and causes the detachment of debris (micro- and nano-particles) from the bulk material (biodestruction) (Didenko et al., 2012; Curia et al., 2014) that will be well documented by scanning and transmission electron microscopy techniques (Figures 2, 3, and 4).

*Figure 1. Scheme of the formation of a cocc albiofilm and polyurethane biodestruction*
1. Initial adhesion of *S. aureus* cells to the polyurethane surface;
2. Formation of microcolonies;
3. Biofilm maturation, appearance of the exopolysaccharide matrix, initial biodestruction of polyurethane and absorption of polymeric nanoparticles by bacterial cells;
4. Detachment of nomadic cells loaded with polyurethane nanoparticles from the mature biofilm.
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