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ELECTRON MICROSCOPIC EXAMINATION OF DENTAL UNIT WATERLINES BIOFILM

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Abstract: Using the transmission electron microscope, the ultrastructural examination was conducted to detect the presence of bacterial biofilm on the inner surfaces of the tubing in dental unit waterlines (DUWL). Samples for examination were taken from the tubes providing water to high-speed and slow-speed handpieces, and to an air-water syringe before application of a disinfection procedure. The microscopic analysis made it possible to find the biofilm in all the tubes in the dental unit which were not pre-disinfected. In these samples, no significant differences were found between high-speed, slow-speed and air-water lines.

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Biofilm is a heterogenic, spatially organised structure, in which microcolonies of one or more microorganisms, exhibiting a definite metabolic activity, are surrounded by particles of extracellular, polysaccharide substance. Biofilm formation consists of the following consecutive stages: adhesion process, forming of microcolonies and of extracellular matrix. The process of microorganisms maturation in the structure of biofilm consists in inducing and suppressing specific genes, and changing the phenotypic properties of sessile cells into the properties characteristic of biofilm mature population. The biofilm can form on virtually every surface remaining in contact with water, among others, on rubber, glass, plastic, and metal. The time of formation and maturation of biofilm, its composition, thickness and properties vary [4, 5, 6, 7, 10, 11, 17].

Dental unit waterlines (DUWL) can contain appoximately 6 m of narrow bore flexible polyurethane or polyvinyl chloride (PVC) plastic tubing with a number of brass couplings, and other non-flexible plastic couplings [16]. DUWL biofilms are adherent colonies of bacteria, fungi, and protozoa that form along the inner surface of dental unit waterlines [1, 2, 10, 11, 15]. The initial biofilm layer thickens through replication of the organisms that make up the biofilm, as well as adherence of free-floating microrganisms from the water source. At times, individual microorganisms, as well as pieces of biofilm, can dislodge and pass out of waterlines. It is at this point that the biofilm becomes a potential problem for dental patient or dental healthcare worker - as a source of microbial contamination [10, 11, 15]. To discover the nature of biofilm in DUWL and the ways to eliminate it, seems a significant research problem. It is known that bacteria adhere more readily to hydrophilic polimeric plastic tubing (polyvinyl chloride, poliuretane) than to the one composed of glass or steel [18].

DUWL biofilm structure and its properties can be studied with various methods, including microscopic examination with the use of an electron microscope, fluorescent microscope, transmission electron microscope, scanning electron microscope (SEM) and confocal laser microscope [10]. These study methods make it possible to

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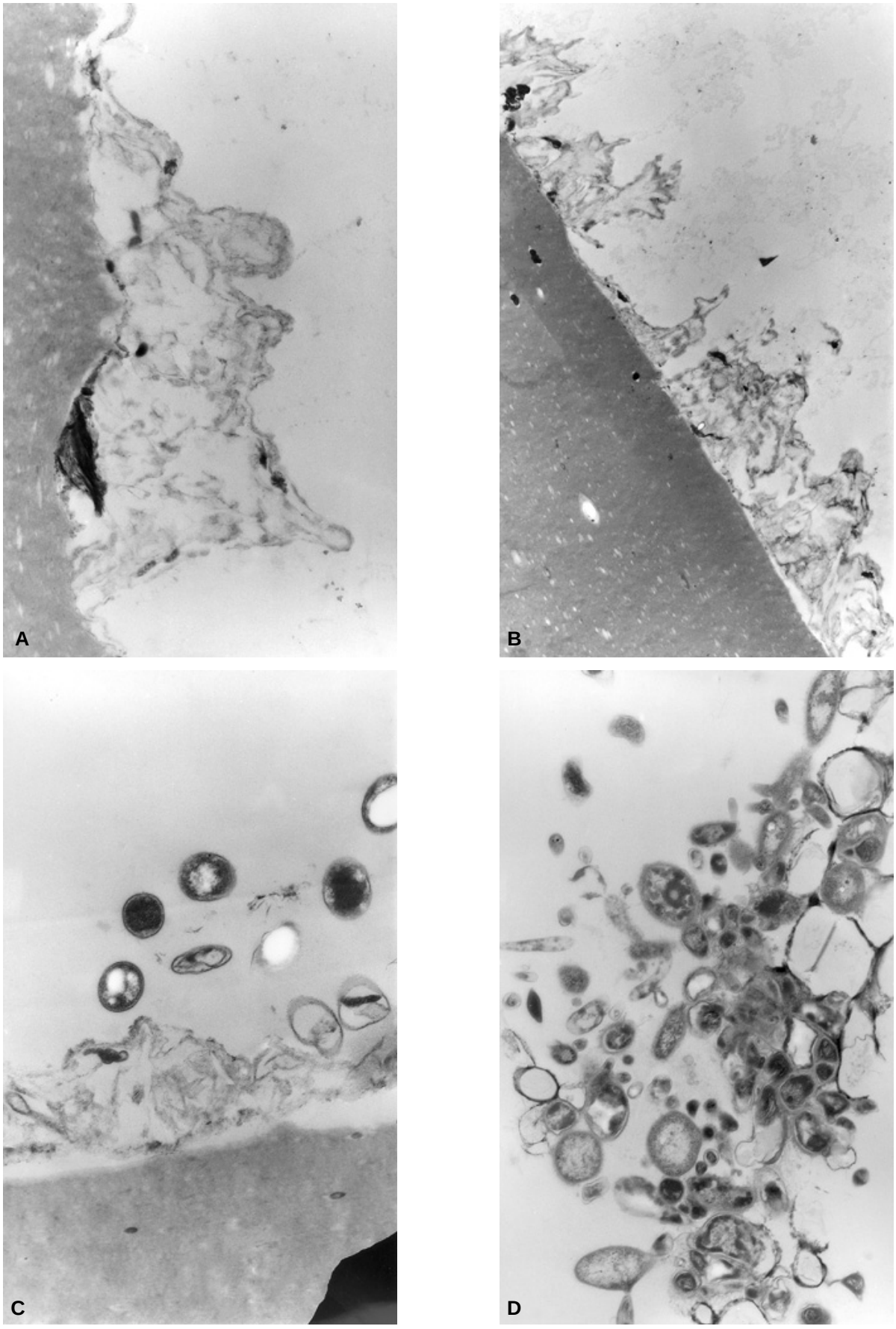
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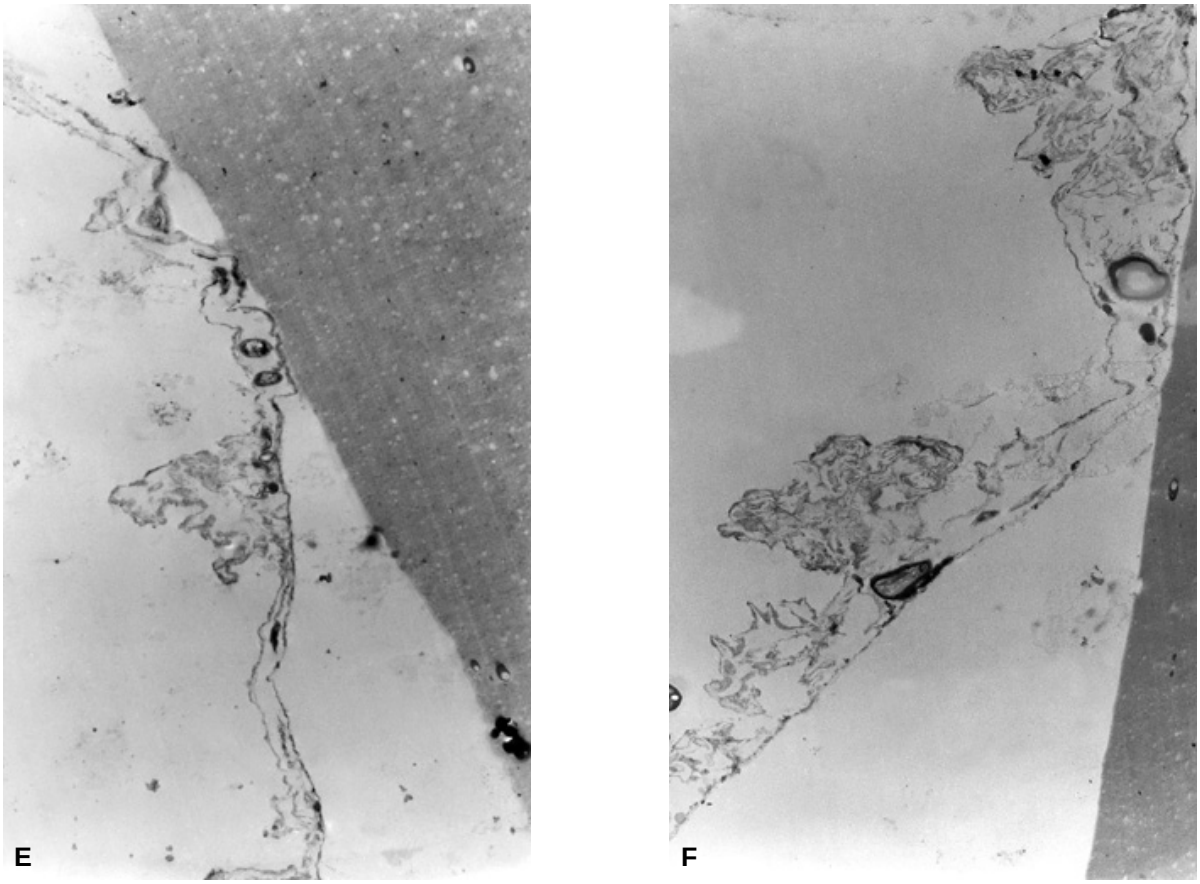

Figure 1. Micrographs of the thin-sectioned DUWL tubes. A) Non pre-disinfected tube providing water to a high-speed handpiece. The biofilm structure and the structures morphologically corresponding to Gram-negative bacteria are present; the structures corresponding to endotoxin-containing microvesicles are visible, ×12,000. B) Non-pre-disinfected tube providing water to a high-speed handpiece. The biofilm present, small mineral elements (needles) are seen, ×6,600. C) Non pre-disinfected tube providing water to a slow-speed handpiece. Bacterial biofilm. Note structures corresponding to protozoa cysts and bacteria, ×18,300. D) Non-pre-disinfected tube providing water to an air-water syringe. A thick mineral layer (probably calcium carbonate deposite by hard water), on which bacteria and protozoa are present. Microcolonies of bacteria are embedded in a dense extracelular envelope, ×14,700. E) Non-pre-disinfected tube providing water to a slow-speed handpiece. Bacterial biofilm present: partly attached to the walls, partly detaching, ×8,000. F) Non pre-disinfected tube providing water to a slow-speed handpiece. Bacterial biofilm present: partly attached to the walls, partly detaching, ×6,600.

find whether the biofilm is present, to show the nature of biofilm, its formation stages, maturation, and also to evaluate the effectiveness of microbial control techniques used for DUWL [1, 2, 3, 8, 9, 12, 13].

MATERIALS AND METHODS

Using the transmission electron microscope, the ultrastructural examination was conducted to detect the presence of bacterial biofilm on the inner surfaces of the tubing in DUWL. The research was carried out at the Electron Microscopy Centre of the Children Hospital at the Jagiellonian University Medical College, Cracow, Poland. Small sections of the tubing were prefixed in 2% glutaraldehyde in phosphate buffer at pH 7.3 and post-fixed in 1% buffered osmium tetroxide. After dehydration in graded series ethanol, the samples were embedded in Low Viscosity (by dr Spurr), thin-sectioned and stained with 2% uranyl acetate and lead citrate. The micrographs were taken with Philips EM 300 electron microscope operating at 80 KV. Attention was paid to the structure and location of the biofilm, the presence of bacteria, other microorganisms, and mineral elements.

Half-thin sections (0.5-1 µm thick) were stained with a mixture of methylene blue and 1% azure II. In the half-thin sections observed under a light microscope, the places for examination in an electron microscope were chosen. The selected places exhibited the presence of a structure resembling the biofilm lining the inner surface of the examined tubes.

Samples for examination were taken from the tubes providing water to high-speed and slow-speed handpieces, and to an air-water syringe before the application of a disinfection procedure.

RESULTS

Figure 1 represent transmission electron micrographs of the thin-sectioned samples of the non-pre-disinfected tube surfaces.

The bacterial biofilm was found on the surface of the tubes providing water to the high-speed handpiece, the

slow-speed handpiece, and the air-water syringe. Structures morphologically corresponding to the colonies of Gram-negative bacteria or endotoxin-containing microvesicles, protozoa cysts, and mineral deposits were seen.

On transmission electron micrographs, both the biofilm attached to the tube walls, and the one detached, could contribute to the contamination of water flowing through the tubing.

CONCLUSION

The microscopic analysis made it possible to find the biofilm in all the tubes in the dental unit which were not-pre-disinfected. In these samples, no significant differences were found between high-speed, slow-speed and air/water lines. The biofilm was found only not on the surface of the disinfected tube.

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