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On the quantification of biocidal activity of protective coatings

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A. Poly(diallyldimethylammonium chloride) (PDADMAC) with an average molecular mass Mw = 470 kDa (CPS Chem. Com. Inc.) was used as a cationic polymer; PDADMAC was dissolved in 10^{-3} M Tris buffer solution with pH7. Concentrations of polymers were expressed in moles of quaternary amino PDADMAC groups [N^+]. To prepare the buffer solution, bidistilled water was used, pre-filtered through a filter with a pore diameter of 20 microns. Also, the finished buffer solution was also filtered using a filter with a pore diameter of 20 microns. Plastic dishes and dispenser tips were pre-treated with UV radiation for 30 minutes. Glassware, metal tweezers, coated glasses and foil used as a substrate for drying glasses were previously sterilized in a medical air sterilizer GP-10 SPU at 160 ºC for 150 minutes. The work used cover glasses with a size of 22x22 mm and a thickness of 0.13 mm (Gerhard Menzel B.V. & Co. KG, Germany).

B. The test cultures used were *S. aureus* ATCC 6538, *P. aeruginosa* 481 and *Y. lipolytica* Y-3322 (VKM). Cultures were grown on agarized lysogenic medium (LB) as modified by Lennox (5 g/L sodium chloride). Cultures were inoculated in dense strokes and left for 24 hours at 30°C in a thermostat. One complete microbial loop (2 mm diameter) of cellular material was removed from the stroke and resuspended in 1 ml of sterile sodium chloride solution (4.5 g/L). The obtained suspensions were further diluted according to experimentally selected dilution values. For *S. aureus*: 50 µl of the initial suspension was added to 950 µl of distilled water (DW); for *P. aeruginosa*: 20 µl to 980 µl of DW; for *Y. lipolytica*: 200 µl to 800 µl of DW. The use of DW

instead of physiologic solution was due to the need to avoid salt crystallization when drying the suspensions.

The obtained suspensions were applied to pre-prepared coverslips: sterile glasses with polymer and degreased glasses without coating. The size of coated glasses was 20x20 mm (area 400 mm^2). The size of the glasses used as controls (uncoated) was 24x24 mm 2 (area 576 mm^2). Degreasing and sterilization of control glasses (Deltalab, Eurotubo #1) was carried out in ultrapure acetone (Komponent-Reactiv, Russia) by immersion method followed by burning in a gas burner flame. The ready diluted suspensions were applied to the surface of cover glasses, containing polymer coating, the volume of the applied suspension was selected experimentally and amounted to 65 µl. For glasses with a larger area (controls) a proportionally increased aliquot of 94 µl was used. The suspensions were applied by the method of fine droplet crushing, distributing the liquid evenly, avoiding uneven distribution of the suspension.

C. To identify viable cells in the initial working suspensions, preliminary studies were performed by staining cells with commercial Live Dead (TF Scientific, USA) according to the kit instructions. To 1 ml of cell suspension was added 3 µl of prepared dye mixture, exposed for 15 minutes in the dark. After that, 5 µl of the suspension was applied to a degreased slide and examined under the microscope using AxioImager fluorescence microscope (Karl Zeiss, Germany) with the light filter set 488024-0000, excitation wavelength 485 and 578 nm; fluorescence in the range of 515-540 nm (green) and 610 nm (red). Photo fixation of the results was performed using a camera and VideoZavr software (Russia). The area of the field of view recorded by the program was 7950 μm^2 when a 100x objective was used (to count bacterial cells) and 13169 μm^2 when a 63x objective was used (counting yeast). Cell counting in photographs was performed manually using the standard PaintBrush application (Microsoft, USA). The number of cells per unit area of glass was recalculated based on the data on the area of the fixed field of view. Counting was performed in 40 fields of view containing up to 200 cells or in 20 fields of view

containing more than 200 cells. Statistical processing of the results and preparation of digital illustrative material was performed using standard MS Excel functions (Microsoft, USA).

To determine the viability of cells plated on the polymer coating, cell suspension was applied to coated and uncoated glasses (control) as described previously, and then the suspensions were dried for 1 hour in a sterile laminar flow box. After drying, the coverslips were placed on slides on which 10 µl of sterile molten agarized LB medium and 5 µl of prepared LiveDead reagent had been previously applied. The drops were mixed and, without waiting for the agar to solidify, the coverslip was placed in a drop of LB medium containing the LiveDead dye mixture, with the surface with the microorganisms in the agar. The polymer layer with the applied cell suspension was fixed in the agarized medium. The slides were then incubated for 15 min in the dark and examined under the microscope as previously described. To detect cell division during daily incubation, a similar technique was used, but no dye was applied. The coverslips were paraffinized along the edge (i.e. filled with a layer of melted paraffin), then microscopy and photo documentation were performed. Then the same slides were placed in Petri dishes with round paper filters moistened with sterile water and left in closed Petri dishes at 30°C for 24 hours. After that, the cells were counted as described above.

D. To determine the adhesion degree of cells to untreated and treated glasses suspensions were applied and dried as previously described. The coverslip was then placed with forceps into a laminar circular flow of sterile distilled water, facing parallel to the flow. The circular flow of liquid was created in a beaker with a volume of 250 ml using a magnetic stirrer (rotation speed of the magnetic stirrer - 240 rpm). The treatment was carried out for 1, 3 and 5 minutes. After that, the glasses were dried. Cell counting was performed according to the protocol previously described.