In vitro diagnostics of microbial cells and their antibiotic resistance using nanostructured anodic aluminum oxide growth platforms

Tatiana Zimina[1], Liudmila Kraeva[2], Ekaterina Sokolova[1], Alexei Soloviev[1], Victor Luchinin[1], Galina Hamdulaeva[2]

[1]St. Petersburg Electrotechnical University "LETI", 5 Professora Popova str., St. Petersburg, Russia
[2]Pasteur Institute of Epidemiology and Microbiology, 14 Mira str., St. Petersburg, Russia
tmzimina@gmail.com

The development of tools for express analysis of human specimens related to aggravated problem of bacteriological threats, combined with the drug resistance is becoming increasingly important. Conventional *in vitro* microbial cells identification and antibiotic resistance diagnosis is based on culture growth of microorganisms. Implementation of this direct approach is usually labour intensive, low throughput and slow. The amplification of clinically relevant DNA by the Polymerase Chain Reaction (PCR) and micro arrays provides data on potential pathogenicity while in clinical use it is crucial to have information on resistance of an actual pathogen from a particular clinical case to correctly select medication. Thus, improving culture based (CB) diagnosis methods could contribute to solving the drug resistance problem, via facilitating the optimal and timely choice of medication. Main limiting factors of CB methods, which restrict their express implementation, are: microorganism growth rate (to make a sample available for manual operations), pathogen recognition and determination of its viability in response to antibiotic exposure (antibiotic susceptibility testing - AST). Miniaturization and heterogeneous integration of living bacteria and MEMS components further contribute to resolving the problems associated with classical CB approaches.

To produce a miniaturised automated platform, appropriate for integration to carry on total microbiological procedure automatically. The presented devise consists of a number of functional modules, namely: colonies growth platform, microfluidic transport system with liquid key for colonies sorting, express pathogen recognition system, AST growth and viability determination module etc. In this work, essential functional elements of the system are presented including culture growth platform (Figure 1) based on porous anodic alumina (PAA), AST diffusion and laser speckle optics turbidimetry modules are discussed. The PAA membranes with regular pore arrays and nano porous supports for the cell growth are fabricated using MEMS/MOEMS technologies. These elements could be incorporated into an integrated system for isolation and reliable direct identification of a range of pathogens, as well as express determination of their viability and threshold concentrations of various classes of antibiotics in the "point-of-care" format. The structured colonies growth platform fabricated on the basis of micro-porous aluminium oxide (AAO) is a principal part of the system[1], which could facilitate direct CB method in terms of analysis time, accuracy and point-of-care availability. The main feature of such platform is a possibility to cultivate homogeneous juvenile colonies, sufficient for identification. Using such juvenile colonies accelerates microbiological analysis by factor of 3 and above as well as provides new opportunities for process automation. Isolated small colonies are grown at spatially arranged growth spots as micropores covered with nanoporous AAO. After limited growth time (lower than in conventional microbiological procedures), when colonies achieve a moderate size of approximately 8 to 128 organisms, they should be cautiously displaced. The separation of juvenile colonies from original "nesting sites" is made by microhydroblow generated from underneath by micro pump. In this case micropores are used as jets to form a hydraulic impulse. Then microflow moves them to the next functional elements. This allows to prepare a sample ready for aligning and transport to other functional elements of total system intended for pathogen identification, sorting and AST. The growth spots are formed in a single technological process of anodic oxidation. The growth platform is intended for further integration targeted at development of full automatic point-of-care microbiological analyser including AST module, with disposable microbial platforms and analysis time 6 – 8 hours. The application of the module in analysis of a number of microbiological samples is described.

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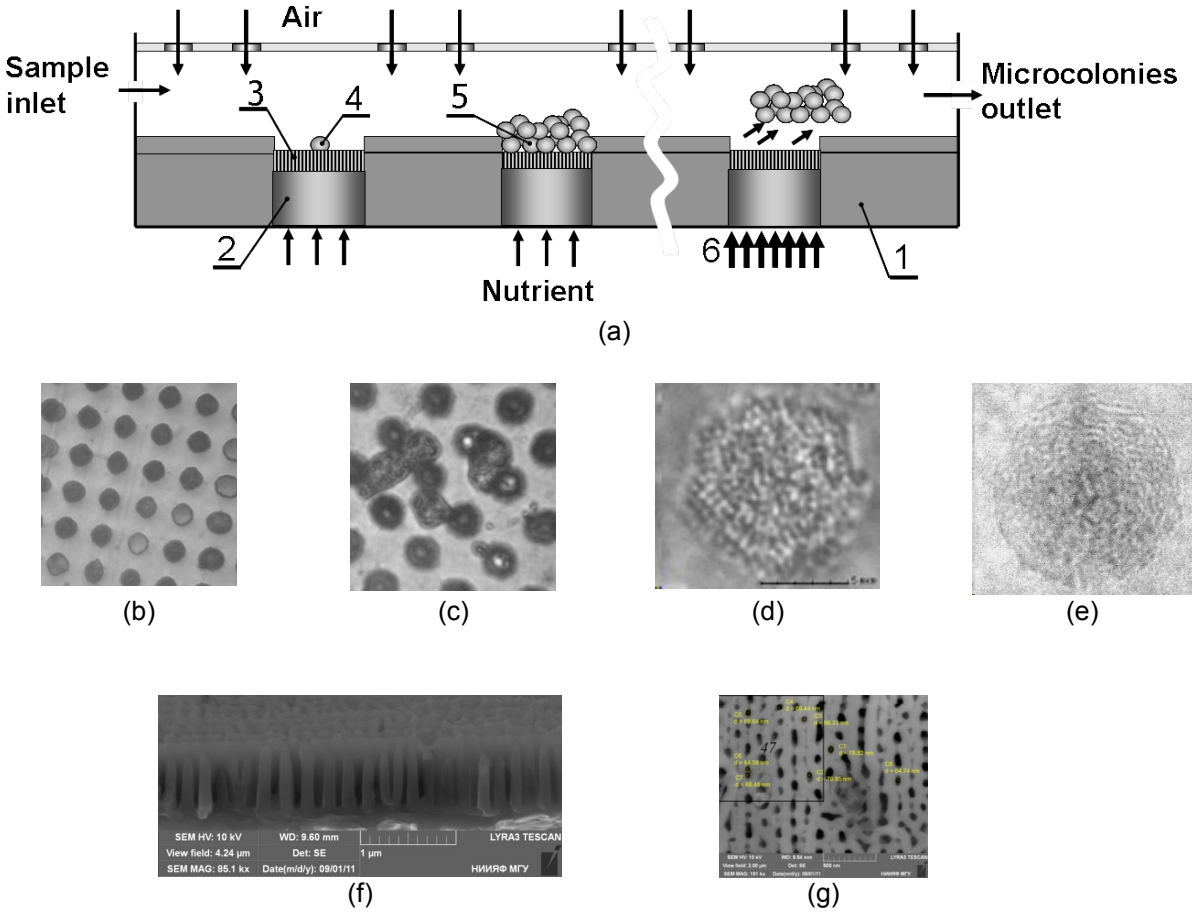

Figure 1 (a) Schematic representation of growth chamber: 1 – AAO substrate, 2 – micropore, 3 – nanoporous Al_2O_3 membrane permeable for nutrient, 4 – cell, 5 – juvenile colony, 6 – direction of microhydroblow displacing colonies; (b) AAO platform with micropores; (c) the same as (b) with microbial cells; (d) juvenile colony of *Staphylococcus aureus,* growth time t = 1 h, number of cells N ~ 800, size of colony ~10 microns, (e) juvenile colony of *Staphylococcus epidermidis*, t = 1 h; (f, g) nanoporous Al_2O_3, section and top view correspondingly.