

## EFFECT OF IMPULSE ELECTRICAL DISCHARGE ON BIOFILM FORMATING ACTIVITY OF THE MICROORGANISMS

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Impulse electrical discharges (IED) induced by generator of device for contact lithotripsy «URAT-1M». The components of electrical discharges are directly acting and destroying the bacteria cell inside the liquid and we extensively used this factor in contaminated surgery. The experiment in vitro shown the inhibition of biofilm forming ability of bacteria under the impact of a subinhibiting dose of IED. This effect is more pronounced for gram negative bacteria. The result of this research is the additional argument to use the methods based on IED for the treatment and prophylaxis of the surgical infection.

**Keywords:** *impulse electrical discharges, biofilm formation activity of bacteria, factors of microorganism's pathogenicity, quorum-sensing of microorganisms, surgical infection*

В качестве генератора импульсных электрических разрядов (ИЭР) применен аппарат для контактной литотрипсии «УРАТ-1М». Компоненты ИЭР в жидкой среде оказывают прямое повреждающее действие на бактериальную клетку, что широко используется нами в гнойной хирургии. В эксперименте in vitro изучена способность бактерий к образованию биопленок в условиях воздействия ИЭР в субингибирующей дозе. Доказан ингибирующий эффект ИЭР разряда на активность микроорганизмов к образованию биопленок. Этот эффект более выражен у грамотрицательных бактерий. Результаты исследования являются дополнительным аргументом в пользу применения методов, основанных на энергии ИЭР, для лечения и профилактики хирургической инфекции.

**Ключевые слова:** *импульсный электрический разряд, биопленкообразующая активность бактерий, факторы патогенности микроорганизмов, кворум-сенсинг микрофлоры, хирургическая инфекция*

### The actuality of the problem

The opportunistic flora of the gastrointestinal tract exhibits prominent force to form biofilms, 64.2% strains has abilities to form the biofilms [1]. Patients has phlegmon of soft tissues of the different parts of the body biofilm formation occurs on 3-5 days after surgery [2]. The authors propose to assess the ability of bacteria to the formation of biofilms because it is a predictor of chronization of disease.

Since in biofilms bacteria are more resistant to the action of antimicrobial agents [3,4], they are resistant to the nonspecific anti-infective factors as well, therefore a method of treatment and prophylaxes of surgical infection must be the suppression of the biofilm formatting ability of the bacteria [5,6]. The biofilm formation in bacteria is one of the factors of pathogenicity that increase the risk of complications after surgery. This factor must be consider especially in the groups of patients with high risk of postoperative complications. Therefore a search for various physical and chemical factors that suppress the biofilm formation activeness of microbes is very important for the treatment and prevention of surgical infection.

In the clinic of Karaganda State Medical University widely used the methods of prevention and treatment of surgical infection, based on the impulse electrical discharge (IED) energy having a multi-component effect on the microflora as well as on the microorganism tissue in purulent [7].

*The aim of research* — to study the effect of the impulse electrical discharge energy on the biofilm formatting of microbes.

### Materials and methods

In the capacity of IED generator the contact lithotripter «URAT-1M» has been used. For the electrodes used coaxial cables, distance between the electrodes are 1.5 mm. Tumbler switch in the scale «5» which corresponds to the maximum impulse frequency.

The micro-organisms selection is on the basis of the following criteria:

— It is known that the causative agents of surgical infection are gram-positive as well as gram-negative flora.

— To increase the authenticity of the results of the microorganisms the used model must be well studied and must also be described.

The standard strains of American Type Culture Collection of Microorganisms were selected — S.aureus ATCC 25923 and E.coli ATCC 25922. Cultures were grown on meat-peptone agar (MPA) for 18-24 hours, prepared by suspension in saline with the concentration of bacterial cells Lg 8 QCU / ml in a volume of 100 ml. To determine the sub-inhibiting effects of IED on the microorganisms, the series of experiments were done in which the duration of IED action was different — from 10 to 300 seconds with intervals of 10 seconds.

For the determination of the biofilm formatting activity the experiment was done by the corresponding method [8]. Measuring the optical density of broth culture was performed at a 540 nm wavelength. For comparison and control the sterile peptide meat broth was used in a similar amount (150 ml), and all manipulations with both (experiment and control) groups were carried out parallel. Indicator of biofilm formation activity expressed as BFA

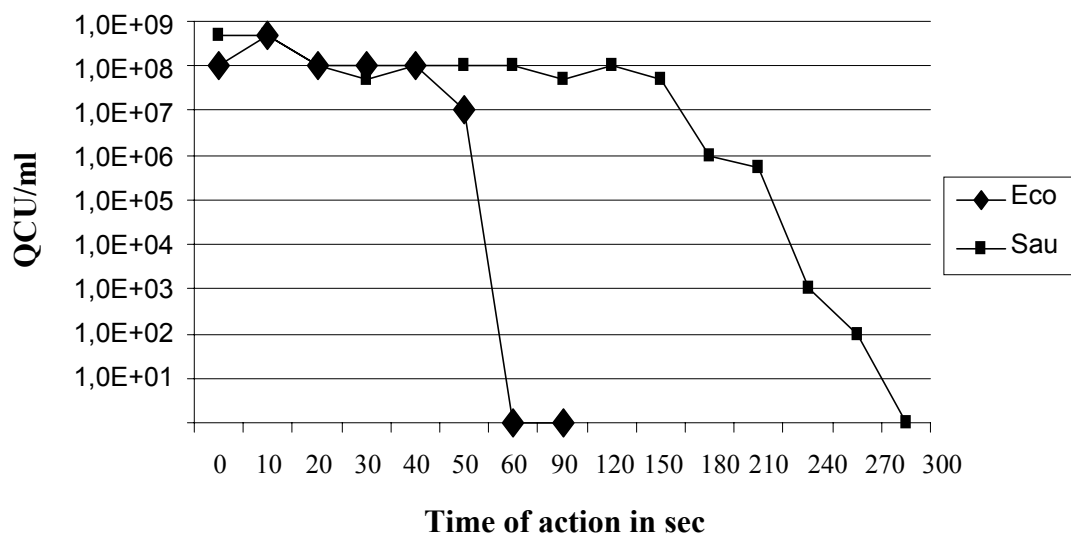


Figure 1. Depend of bacterial QCU from exposure time of IED action

(Biofilm Forming Activity), in arbitrary units (arb.unit) and calculated by the formula (1):

$$BFA = (A_{540 \text{ experiment}} - A_{540 \text{ experiment zero}}) : (A_{540 \text{ control}} - A_{540 \text{ control zero}}); \quad (1)$$

where:  $A_{540 \text{ experiment}}$  — value arb.unit optical density obtained when measuring in the test socket after the formation of biofilms;  $A_{540 \text{ experiment zero}}$  — the value of arb.unit optical density obtained when measuring in the unsown experiment socket until the formation of biofilms;  $A_{540 \text{ control}}$  — value arb.unit optical density obtained when measuring in the control socket after the formation of biofilms;  $A_{540 \text{ control zero}}$  — the value of arb.unit optical density obtained when measuring in the unsown control socket until the formation of biofilms.

Strains to be considered biofilm formable for when the values of BFA more than 1.1 arb.unit [9].

### The results

The study of the IED upon the model of microorganisms has shown that gram-negative bacteria (*E.coli*) are less resistant to the action than *S.aureus*. The *E.coli* titer remained at baseline 8 Lg QCU/ml after the exposure for 40 seconds. In exposition for 40 sec the titer *E.coli* remain on the level 8 Lg QCU/ml. With the increase of IED's action time the titer *E.coli* suddenly decreased and after 60 sec exposition the titer of viable bacteria was contain less then 1 Lg QCU/ml.

With increasing the time of exposure upon the Gram-positive microorganisms (*S.aureus*) to 150 seconds the preservation of the original titer was 8 Lg QCU/ml. Further increase in exposure time caused a gradual decrease in EDI caused the decreased in titer of bacteria, and exposure 300 seconds titer of remained viable *S.aureus* was less than 1 Lg QCU/ml (Fig.1).

Thus, for example on *E.coli* to determine the impact on the ability of biofilm formation as a subinhibition regime was taken 40 seconds of IED action.

Studies indicate that both model of microorganism has biofilm formatting potential (Fig.2), while even before the impact of IED *E.coli* had a less pronounced ability to form biofilms (1.19 BFA) vs. with *S.aureus* (2.76 BFA).

After IED during 40 seconds the biofilm formatting ability was reduced in both model of organisms. The Gram-negative bacteria (*E.coli*) having initially low ability to form biofilms this pathogenicity factor were inhibit up to complete disappearance (Fig. 2).

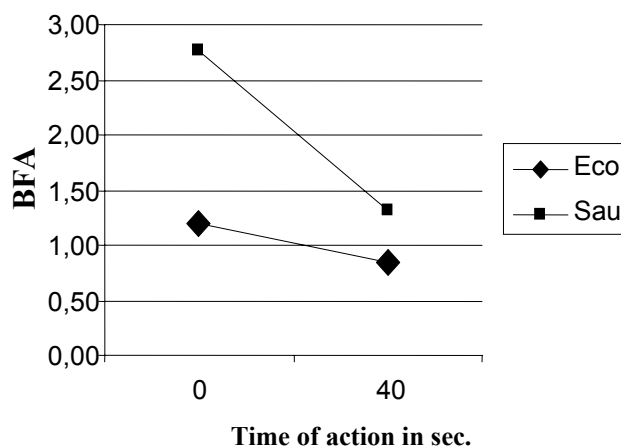


Figure 2. Biofilm formation activity model strains microorganisms after IED. The thick line marked threshold 1.1 BFA

After the IED action biofilm formation activeness was lower than in the control and reached 0.83 BFA, the same number of viable cells after exposure to IED is comparable to the control values (8 Lg QCU/ml). These features indicate a change in the qualitative characteristics of bacterial cells, especially — to reduce the ability of cells to contact with each other and form a biofilm, i.e., occurs their disorientation. Under the influence of IED in the Gram-positive microorganisms (*S.aureus*) were also decreased biofilm formatting activeness (up to 1.31 BFA), while maintaining the number of viable cells at the level of control. It is known that biofilm formation are regulated by «quorum sensing» in bacteria [10]. Consequently, we can assume that the impact of IED violates not only the integrity of bacterial cell walls, but also leads to disorientation of isolated bacterial cells, disrupting «Quorum Sensing» mechanism.

The violation of this mechanism leads to slow the formation of biofilms and reduces the risk of postoperative complications, which is an additional argument to use methods based on IED in clinical practice for prevention and treatment of surgical infection.

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