ABILITY TO FORM BIOFILMS BY PYELONEPHRITIS CAUSATIVE AGENTS IN CHILDREN

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The course of infectious diseases can proceed with complications just because of the formation of microbial biofilms in organism. Many chronic diseases, such as urinary tract infections including pyelonephritis, are associated with biofilm infections. [1,2]. Planktonic bacteria are known to reach the kidneys in an ascending way and be able to attach to uroepithelium and kidney papillae in kidney collective systems. Bacterial adhesion is the main moment in tissue surface of host organism. Adhesion of microorganisms to uroepithelium enables them to resist removal by urine flow. Bacterial adhesion not only promotes colonization, but also favors invasion of microorganisms, biofilms formation and damage of host cells with pyelonephritis development. Biofilms on uroepithelium surface are easy eradicated by antimicrobial agents compared with biofilms formed on alien objects in urinary system. Alien body in urinary tract (catheters, stents, drainages, stones) becomes the infection source for the organism which leads to the development of urinary system complicated infections [3,4]. Periodic release of bacteria planktonic forms from biofilms with urine flow is the source of maintenance of chronic infections and inflammatory process in kidneys.

The biofilms study nowadays attracts great interest of the researches mainly for the reason that this way of bacteria existence creates more problems in medical practice. Biofilms present one of pathogenic factors of pyelonephritis chronic forms formation [5, 6].

Bacteria living in biofilms significantly differ from planktonic forms in their biological properties. Bacteria stability in biofilms to antibiotics and attack of immune system draws more attention. The nature of this stability is intensely studied at present. Thus, the ability of biofilms bacteria to survive in presence of antibiotics in concentrations sufficiently exceeding standard therapeutic concentrations creates difficulties in pyelonephritic treatment in children as this poses production of polyresistant planktonic cells the result of which is the appearance of chronic pyelonephritis and frequent relapses [7].

The problem of suppression and destruction of bacterial biofilms is the extremely urgent task as in clinics classical methods of antibiotic therapy of purulent-inflammatory infections are often ineffective and unpredictable because of high resistance of causative agents in biofilms.

Thus, at present means and methods allowing the destruction of biofilms and facilitating access of antimicrobial preparations to planktonic cells are actively sought. Thorough study of biofilms formation processes by pyelonephritis causative agents with the help of light, fluorescent and scanning microscopy is necessary [8].

The aim of the given study was the detection of the ability to biofilms formation by bacteria, pyelonephritis causative agents in children with the help of light, fluorescent and scanning microscopy.

Material and methods. Bacteriological method for microorganism identification according to accepted microbiological schemes of microorganisms allocation and identification was used to achieve the goal [9]. Sterile polymeric Petri dishes d=40 mm were used to receive biofilms. Each dish was placed 4 ml of Müller-Hinton broth and daily microorganisms culture excreted from urine in children with pyelonephritis and incubated for 12-24 hours under +37°C. After incubation the growth media was poured out, the dishes were rinsed twice by Hencs solution (2 ml), fixed by 10% solution of formalin on distilled water (pH=7,2-7,4), dried, stained by 1% solution of crystal violet and washed by distilled water [10].

The preparations microscopy was done with the help of Granum microscope with oil immersion. Bacteria and their biofilms digital imaging was received with the help of ToupCam 3.1 MP video camera (video ocular) and saved in jpeg format.

Scanning (the department of experimental physics of the physical faculty of V.N.Karazin KNU) and fluorescent microscopy (for daily biofilms staining 200 mcl of working mixture of acridine orange (concentration 2 mcg/ml) were used for visualization of biofilms morphologic structure in high resolution. The records were kept with the help of fluorescent microscope, enlargement 100x10x1,5, using filters, providing exciting light with the wave length not more than 490 nm and emission with the wave length 520 nm).

Fig. 1. Determination of biofilm formation ability with the help of light, fluorescent and scanning microscopy (I – light microscope; II – fluorescent and scanning microscope)

Results and their discussion. The study demonstrated that all isolates formed biofilms. Adhesion of planktonic bacteria forms took place on the first stage, intracellular matrix formation occurred on the second stage and biofilm formation took place on the third stage. During the study of *E. coli* and *Proteus spp.* bacteria preparations with the use of scanning and light microscopy ordered bacteria arrangement was seen in the form of separate structures or tiny clusters of bacterial cells united by matrix (Fig. 2, 3).

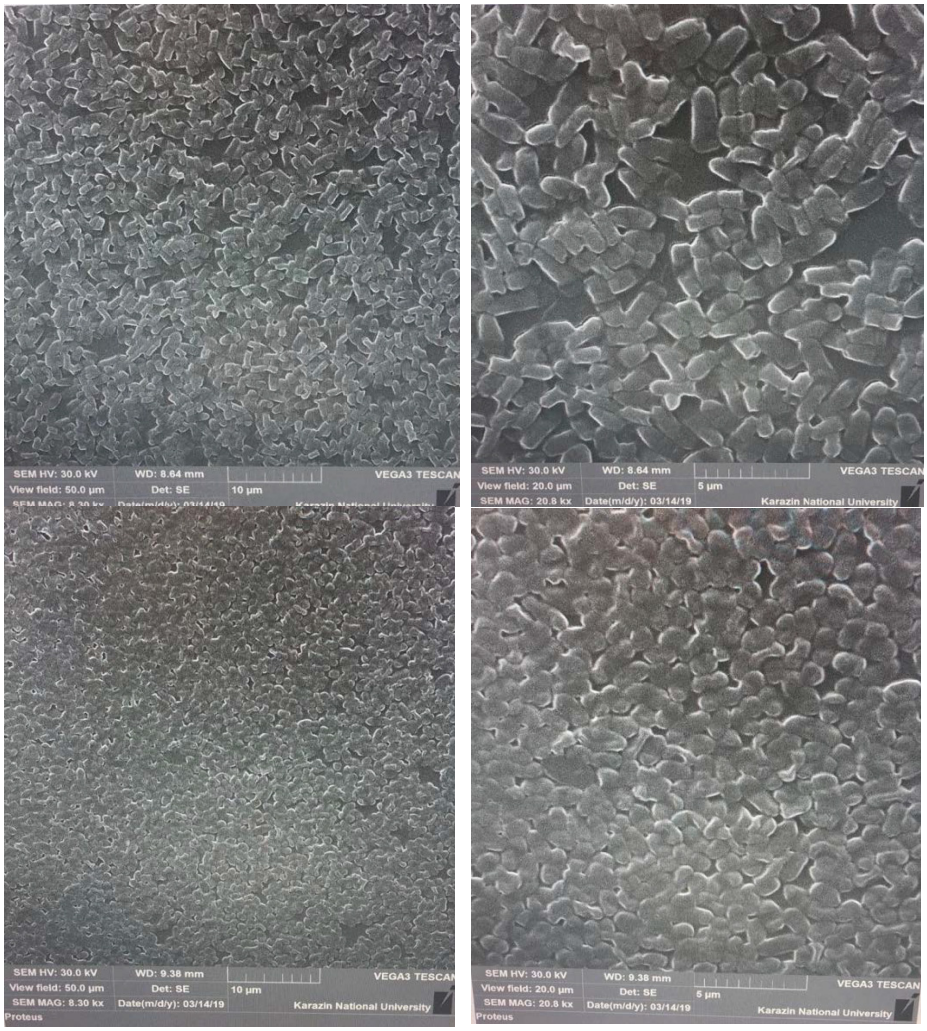

Fig. 2. Bacteria biofilms formation: 1 – E. coli; 2 – Proteus spp.; scanning microscopy

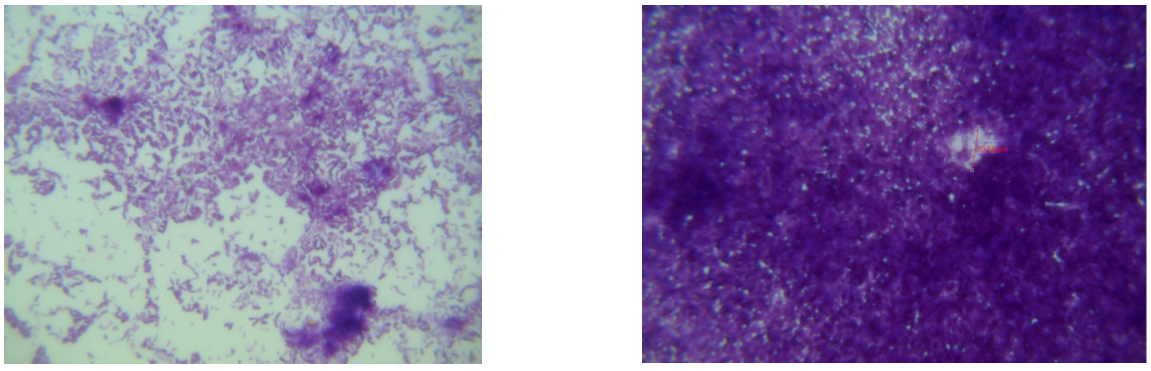

Fig. 3. E. coli isolates biofilms formation:1 – with acute form of pyelonephritis; 2 – with chronic form of pyelonephritis. Light microscopy

During the study of the ability to form *P. aeruginosa* isolates biofilms with the help of scanning microscopy it was stated that the adhesion of separate bacterial cells occurs with further conglomerates formation which are surrounded by matrix with further biofilm formation (Fig. 4). Packed biofilms areas with cells clusters with good fluorescence were found with the help of fluorescent microscopy (Fig. 5).

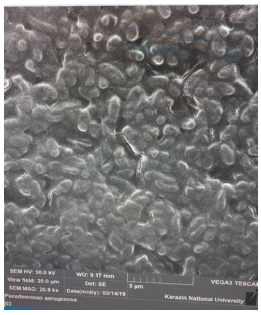

Fig. 4. P. aeruginosa bacteria biofilms formation.; scanning microscopy

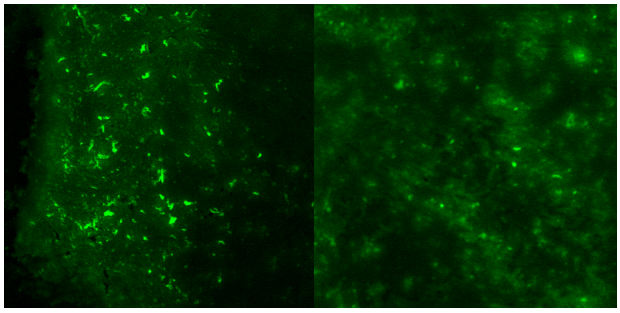

Fig. 5. Ability to P. aeruginosa bacteria biofilms formation; fluorescent microscopy

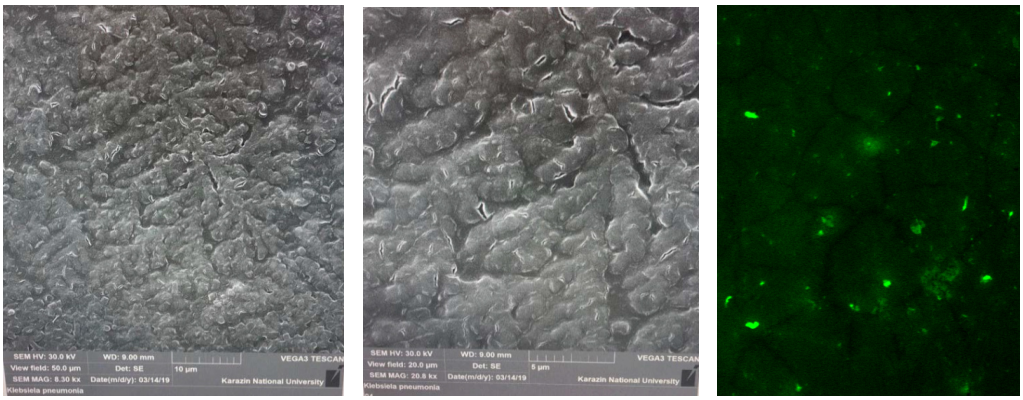

Fig. 6. K.pneumoniae bacteria biofilms formation; scanning and fluorescent microscopy

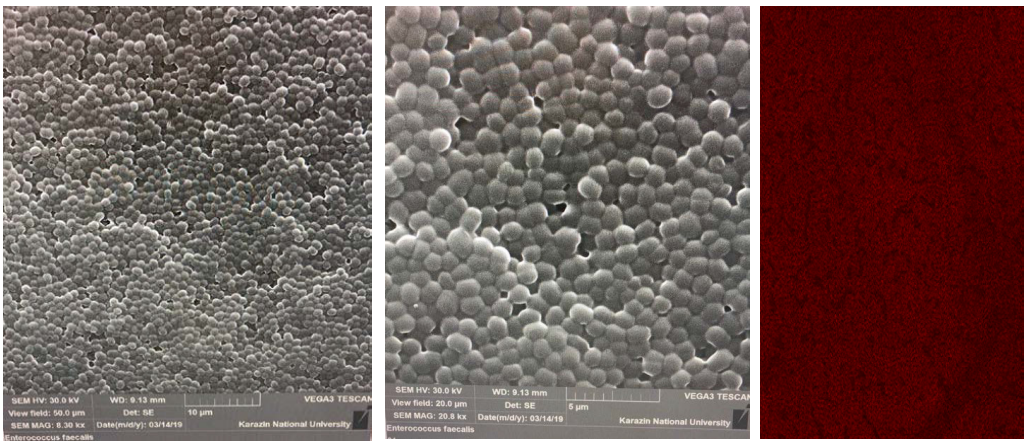

Fig. 7. E. Faecalis bacteria biofilms formation; scanning and fluorescent microscopy

During daily *K.pneumoniae* isolates biofilms study by methods of scanning and fluorescent microscopy (Fig. 6) it was found by that *K.pneumoniae* biofilms were covered with dense matrix and riddled with multiple canals in the form of apertures.

During morphological peculiarities study of *E. faecalis* isolates biofilms formation with the use of scanning and fluorescent microscopy it was found that bacterial cells were densely packed and united by intracellular matrix under which bacteria of spherical shape were seen. (Fig. 7).

Thus biofilms, the nature of which depends on the type of bacteria, are formed on the surface of conglomerates consisting of bacterial cells. Peculiarities of course and appearance of pyelonephritis chronic form and relapses in children is explained by biofilms formation.

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SUMMARY

ABILITY TO FORM BIOFILMS BY PYELONEPHRITIS CAUSATIVE AGENTS IN CHILDREN

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The work is dedicated to the study of biofilms formation process by main pyelonephritis causative agents in children *in vitro* using methods of light, fluorescent and scanning microscopy. To study biofilms formation bacteria were cultivated in liquid substratum on glass in polystyrene Petri dishes d=40mm. The study demonstrated that all isolates formed biofilms. Adhesion of bacteria planktonic forms took place on the first stage, intracellular matrix formation took place on the second stage, and biofilms formation took place on the third stage.

During the study of *E. coli* and *Proteus spp* bacteria preparations with the use of scanning and light microscopy ordered bacteria arrangement was seen in the form of separate structures or tiny clusters of bacterial cells united by matrix. During the study of the ability to form *P. aeruginosa* isolates biofilms with the help of scanning microscopy it was stated that the adhesion of separate bacterial cells occurs by conglomerates formation surrounded by matrix with further biofilms formation. Bacterial cells in the form of dense elongated sticks were seen under the film. *P.* a*eruginosa* isolates daily biofilms were stated to have dense structure in the form of gel. Packed biofilms areas with cells clusters with good fluorescence were found with the help of fluorescent microscopy. During daily *K.pneumoniae* isolates biofilms study by methods of scanning and fluorescent microscopy it was found that *K.pneumoniae* biofilms were covered with dense matrix and riddled with multiple canals in the form of apertures. During morphological peculiarities study of *E. faecalis* isolates biofilms formation with the use of scanning and fluorescent microscopy it was found that bacterial cells were densely packed and united by intracellular matrix under which bacteria of spherical shape were seen.

Thus biofilms, the nature of which depends on the type of bacteria, are formed on the surface of conglomerates consisting of bacterial cells. Peculiarities of course and appearance of pyelonephritis chronic form and relapses in children is explained by biofilms formation.

Keywords: bacteria biofilms, isolates, scanning, light, fluorescent microscopy, pyelonephritis in children.

РЕЗЮМЕ

СПОСОБНОСТЬ К ОБРАЗОВАНИЮ БИОПЛЕНОК ВОЗБУДИТЕЛЯМИ ПИЕЛОНЕФРИТОВ У ДЕТЕЙ

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Статья посвящена исследованию процесса формирования биопленок основными возбудителями пиелонефритов у детей *in vitro* с использованием методов световой, люминесцентной и сканирующей микроскопии. В результате исследования установлено, что все изоляты образовывали биопленки.

Выявлено упорядоченное расположение изолятов *E. coli* и *Proteus spp.* в виде отдельных структур или небольшого скопления бактериальных клеток, объединенных матриксом. Установлено, что адгезия отдельных бактериальных клеток *P. aeruginosa* происходит с формированием конгломератов, окруженных матриксом с последующим образованием биопленки.

Выявлено, что суточные биопленки изолятов *P. aeruginosa* имеют плотную структуру в виде геля, биопленки *K.pneumoniae* покрыты плотным матриксом и пронизаны множественными каналами в виде отверстий, изоляты *E. faecalis* плотно упакованы и объединены межклеточным матриксом, под которым видны бактерии шаровидной формы.

Таким образом, на поверхности конгломератов, состоящих из бактериальных клеток, формируются биопленки, природа которых зависит от вида бактерий. Формированием биопленок объясняются особенности течения и возникновения хронической формы и рецидивов пиелонефрита у детей.

რეზიუმე

ბიოაპკების წარმოქმნის უნარი პიელონეფრიტის გამომწვევებში ბავშვებში

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ხარკოვის ეროვნული სამედიცინო უნივერსიტეტი, [1]დ. გრინევის სახ. მიკრობიოლოგიის, ვირუსოლოგიისა და იმუნოლოგიის კათედრა; [2]პედიატრიის №2 კათედრა; [3]ხარკოვის ვ.კარაზინის სახ. ეროვნული უნივერსიტეტი, კლინიური იმუნოლოგიისა და ალერგოლოგიის კათედრა, უკარინა

ნაშრომი ეძღვნება პიელონეფრიტის ძირითადი გამომწვევების მიერ ბიოაპკების წარმოქმნის პროცესის *in vitro* კვლევას ბავშვებში სინათლის,ლუმინესცენტური და მასკანირებელი მიკროსკოპიის მეთოდების გამოყენებით. კვლევის შედეგად დადგენილია, რომ ბიოაპკებს წარმოქმნის ყველა იზოლატი. გამოვლენილია *E. coli* და *Proteus spp.*-ის იზოლატების თანმიმდევრული განაწილება ცალკეული სტრუქტურების ან მატრიქსით გაერთიანებული ბაქტერიული უჯრედების მცირე დაჯგუფებების სახით. დადგენილია, რომ *P. aeruginosa*-ას ცალკეული ბაქტერიული უჯრედის ადჰეზია ხორციელდება მატრიქსით გარშემორტყმული კონგლომერატების ფორმირებით, ბიოაპკის შემდგომი წარმოქმნით. გამოვლენილია, რომ *P. aeruginosa*-ას იზოლატების დღეღამურ ბიოაპკებს აქვს მკვრივი სტრუქტურა გელის სახით,*K.pneumoniae* -ს ბიოაპკები დაფარულია მკვრივი მატრიქსით და ნახვრეტების სახით მრავალრიცხოვანი არხებითაა გამსჭვალული, *E. Faecalis*-ის იზოლატები მკვრივადაა შეკრული და გაერთიანებული უჯრედშორისი მატრიქსით, რომლის ქვეშაც აღინიშნება სფეროს ფორმის ბაქტერიები.

ამრიგად, ბაქტერიული უჯრედებისაგან შემდგარი კონგლომერატების ზედაპირზე ფორმირდება ბიოაპკები, რომელთა თვისებები ბაქტერიების სახეობაზეა დამოკიდებული. ბიოაპკების ფორმირებით აიხსნება პიელონეფრიტის მიმდინარეობის, ქრონიკული ფორმების და რეციდივების განვითარების თავისებურებები ბავშვებში.

ДИНАМИКА ИЗМЕНЕНИЙ УЛЬТРАСТРУКТУРЫ МАКРОФАГОЦИТОВ РАНЕВОГО КАНАЛА ПОСЛЕ ОГНЕСТРЕЛЬНОГО РАНЕНИЯ

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Несмотря на стремительное развитие современных средств поражения во время локальных военных конфликтов, миротворческих миссий и военных операций, наибольшее количество повреждений наносится огнестрельным оружием [4,17,19].

В структуре огнестрельных ранений, полученных во время вооруженных конфликтов, превалируют травмы конечностей с повреждением костей и мягких тканей. По результатам анализа случаев ранений американских военнослужащих во время второй мировой, вьетнамской, иракской войны, доля проникающих ранений конечностей составляет от 50% до 70%, причем только у трети пострадавших это были пулевые ранения [2,5,13].

По данным J.J. Doucet и соавт. [8], боевая травма, в отличие от гражданской, в большинстве случаев (в 63,3%) повреждает нижние конечности, в основном, в результате взрывов, в 16,3% - от высокоскоростных огнестрельных ранений.

Несмотря на многовековую историю применения огнестрельного оружия, углубление данных патогенеза огнестрельных ран и множества предложенных методик оказания медицинской помощи [10,11,16,18] вопросы длительности восстановления мягких тканей после огнестрельных осколочных ранений по сей день не изучены [1,15].

Недостаточно изучен вопрос длительности изменений и полноценности восстановления макрофагальной системы рубцовых тканей после огнестрельных осколочных ранений. Одним из методов, позволяющих оценить восстановление функционирования и структуры тканей на субклеточном уровне является электронная микроскопия [3,6,14].

Цель исследования - выявить особенности перестройки субмикроскопической архитектоники макрофагоцитов скелетных мышц, динамику трансформаций органелл и внутриклеточных мембран в различные сроки после моделированного огнестрельного осколочного ранения.

Материал и методы. Для реализации поставленной цели исследования использованы 26 лабораторных животных – племенных кроликов породы «Шиншилла», с массой тела 2200-3000 г. Средняя масса животных составила 2620±120 г. За 20 минут до начала экспериментов проводили обезболивание лабораторных животных препаратом налбуфин в дозе 0,3 мг/кг массы тела животного, внутримышечно. Лабораторным животным наносили огнестрельное ранение в области мышц бедра из пистолета «Форт-12» калибр 9 мм, с усиленным патроном, заряженным обрезанными (без шляпки) металлическими шурупами СМК 3,5х9,5 («саморез») массой 0,9-1,1 гр с дистанции 3,0 м. Начальная скорость осколка составила 305 м/сек. Моделирование огнестрель-