ISSN: 0975-766X
CODEN: IJPTFI

Available Online through ***Research Article***
www.ijptonline.com

STUDY OF SENSITIVITY OF MICROORGANISMS ISOLATED FROM DUCTAL BILE OF PATIENTS WITH CHOLEDOCHOLITHIASIS AND ACUTE SUPPURATIVE CHOLANGITIS TO THE CARBON-SILVER COATINGS

Vladimir F. Kulikovsky, Alexander V. Soloshenko, Andrei L. Iarosh, Alexander A. Karpachev, Sergei B. Nikolaev, Yurii Y. Vlasuk, Boris V. Kasyanov, Angela V. Gnashko.
Belgorod State University, Pobeda str. 85, Belgorod, 308015, Russia,
Email: yarosh_a@bsu.edu.ru

Received on 15-10-2016 *Accepted on 18-11-2016*

Abstract

In the study was examined the sensitivity of the microorganisms isolated from the ductal bile of the patients with choledocholithiasis and acute suppurative cholangitis to the elements of the silver endobiliary stents's carbon coating. Microorganism strains of Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli and Klebsiella pneumoniae were sensitive to the coating elements - the zones of inhibition around the test samples were from 12.3 ± 1.43 mm in Enterobacter cloacae to 24.3 ± 1.45 in Escherichia coli. Pseudomonas aeruginosa strains were completely not susceptible to cover elements – it was presented by the absence of zones of growth inhibition around the test samples. The sensitivity of anaerobic strains such as Bacteroides fragilis and Peptostreptococcus anaerobius to the cover elements revealed sufficiently stable zones of delay of microorganisms 16.5 ± 2.78 and 21.5 ± 1.78 mm respectively. Effects of silver nanoparticles in bacterial cell adhesion stadium reduces the colonization and growth of bacterial cells communities, thereby showing the susceptibility of microorganisms isolated from the ductal bile of the patients with choledocholithiasis and purulent cholangitis, silver-carbon elements to the coating.

Keywords: stent, biofilm, choledocholithiasis, cholangitis.

Introduction

One of the actual today's problems is the problem of surgical treatment of obstructive jaundice caused by gallstones (Parfenov, I.P, Iarosh, A.L, Soloshenko, A.V, Karpachev A.A, Sergeev OS., 2011; Copelan, A, Kapoor, B.S. 2015; Wandling, M.W, Hungness, E.S, Pavey, E.S et al., 2016).

The constant introduction of new methods of diagnostics does not reduce the amount of severe cases and patients still continue to arrive in surgical hospitals with severe obstructive jaundice. One of the main treatments for obstructive jaundice currently is endoscopic transpapillary stenting of the bile ducts. The main disadvantage is the need to replace

the stent after 3-4 months - plastic stents because of their lumen obstruction by bile salts, due to the formation of the protein film on the surface of endobiliary prosthesis, to which substrate occurs the adhesion of the bacterial cells, after the colonization of microorganisms, deposition of calcium salts and eventually occlusion of the lumen of the endoprosthesis. In scientific circles the question of the effectiveness of bile flow is widely discussed, while there is no consideration about the work prolongation technologies of the of plastic stents (Kotovsky A.E, Glebov K.G, 2008; Dyuzheva, T.G, Savitskaya, E.E, Kotovsky A.E, et al., 2012; Siiki A., Helminen M., Sand J., Laukkarinen J., 2014; Lebeaux D., Ghigo J.M, Lucet J.C, 2014).

One of the promising nanoscale coatings is a diamond-like carbon coating with silver nanoclusters. The impact of silver nanoparticles on bacterial cell in adhesion stage reduces the colonization and growth of bacterial cells communities, which will extend the life of biliary stent s and reduce the development of septic complications in the postoperative period (Katsikogianni, M, Spiliopoulou, I, Dowling D.P, Missirlis, Y.F. 2006; Zong-ming Xiu et al., 2012; Lebeaux, D., J. M. Ghigo, J. C. Lucet, 2014; Kulikovskii, V.F, Soloshenko, A.V, Iarosh, A.L et al., 2015).

Materials and Methods

Creation of the experimental samples of the biliary stents was performed in the laboratory "Problems of development and introduction of ion-plasma technologies" in "Belgorod State University". For this purpose, the plastic stent was applied with nanoscale diamond-like carbon coating including silver nanoclusters.

The choice of this type of coverage is due to the carbon of properties: high hardness, low coefficient of friction, chemical inertness, optical characteristics and biological inertness.

We used a pulsed vacuum-arc sputtering method with a target of graphite in an electric discharge with turning into graphite carbon plasma followed by deposition on a cold substrate. In addition, by adjusting the pulse repetition frequency, it is possible to keep the temperature as low as possible, which allows coating not heat-resistant materials, including polymers. Thus, the coating thickness may range from 10 nm to 2.0 microns. Figure 1 shows an electron microscope image of a diamond-like carbon film of 80 nm thick in "transmission" mode.

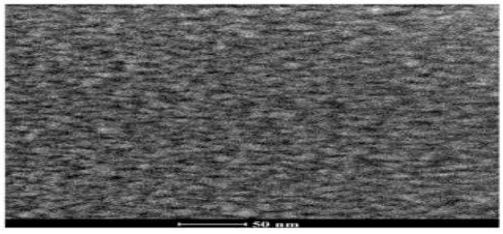

Fig. 1. The electronic and microscopic image of a carbon diamond-like film 80 nanometers thick in the mode "on a gleam" (× 2000).

In order to study the possibility of long-term internal drainage of the bile duct in patients with unresolved choledocholithiasis, including complicated by acute suppurative cholangitis, experimental samples studied groups of stents were incubated for 90 days at a temperature 36.6°C in bile obtained from patients with choledocholithiasis, including complicated by cholangitis.

Every three days in medical bile flask was changed to "fresh", the samples were removed and the stents were washed with sterile saline. To assess the sensitivity of microorganisms to the studied coating using Mueller-Hinton Agar (aerobic microorganisms) and Anaerobic Agar Base of the firm Himedia (anaerobic microorganisms). We used nosocomial multiresistant strains of bacteria isolated from the bile of patients with choledocholithiasis, which were complicated by purulent cholangitis: Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Bacteroides fragilis and Peptostreptococcus anaerobius.

Strains were identified using standard methods and biochemical tests to identify the commercial systems API 20E, API 20NE (bio Merieux, France).

We have tested nanosized diamond-like carbon coating with silver nanoclusters, as a control using traditional biliary uncoated stent.

The test materials had the same surface area. Studies carried out during the day when cultured under aerobic conditions at a thermostat 36°C (for aerobes) and 37°C (for anaerobes). To create anaerobic conditions used Anaero Jar (OXOID). Microorganisms were plated in the standard concentrations "lawn" on agar plates, and then laid on the agar surface material. The area (mm) of growth inhibition around the test samples colonies were recorded after 16-20 hours.

Results and Discussion.

We studied the sensitivity of the microorganisms isolated from the ductal bile of patients with choledocholithiasis and acute cholangitis (Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacteroides fragilis, Peptostreptococcus anaerobius) to the elements of the carbon coating c inclusion of silver nanoclusters. The diameters of the zones of microbial growth inhibition are presented in Table 1.

The table shows that the material is uncoated (control), made no effect on the reproduction of microorganisms that was characterized by the absence of delays of the colony growth around the test samples. A different picture emerges

from a study of the sensitivity of microorganisms to the elements of nanosized diamond-like carbon coating with silver nanoclusters.

Table 1: The diameter range of inhibition zones of microorganisms isolated from ductal bile of the patients with choledocholithiasis and purulent cholangitis to the elements of the carbon coating with silver nanoclusters.

The character of the coating	**Enterobacter aerogenes**	**Enterobacter cloacae**	**Escherichia coli**	**Pseudomonas aeruginosa**	**Klebsiella pneumoniae**	**Bacteroides fragilis**	**Peptostreptococcus anaerobius**
Control (material without coating)	0	0	0	0	0	0	0
Nanosized diamond-like carbon coating with silver particles	15.5±1.78	12.3±1.43	24.3±1.45	0	18.3±1.67	16.5±2.78	21.5±1.78

Studies have shown that nanosized diamond-like carbon coating with the inclusion of nanoclusters with the silver deposited on the surface of the endobiliary stent has bactericidal activity against strains of Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Bacteroides fragilis and Peptostreptococcus anaerobius, that were isolated from the ductal bile of the patients with choledocholithiasis and purulent cholangitis. It was characterized by reliable retardation zones of growth of previously discussed colonies of microorganisms around test samples. It is known that bacterial adhesion and biofilm formation on the surface of biliary stents play a major role in the mechanism of early obstruction (Dyuzheva TG et al, 2012;. Kotovsky AE, Glebov KG., 2008). All of this allows us to assume that the nanosized diamond-like carbon coating with silver nanoclusters will significantly extend the life of endobiliary stents and reduce the risk of septic complications (Xiu, ZM., Zhang, Q.B.; Puppala H.L. et al., 2012.).

Conclusion: In the research of the sensitivity of the microorganisms isolated from the ductal bile of the patients with choledocholithiasis and acute suppurative cholangitis, the elements of a nano-sized diamond-like carbon coating with the inclusion of silver nanoclusters, revealed distinct zone of inhibition of colonies around the test samples (Enterobacter aerogenes – 15.5 ± 1.78 mm, of Enterobacter cloacae – 12.3 ± 1.43 mm, Escherichia coli – 24.3 ± 1.45 mm, Klebsiella pneumoniae – 18.3 ± 1.67 mm, Bacteroides fragilis – 16.5 ± 2.78 mm, Peptostreptococcus anaerobius - 21.5 ± 1.78 mm). Other materials studied (control) didn't affect the reproduction of the microorganisms, that was characterized by the absence of the colony growth delay around the test samples.

References.

1. Copelan, A, Kapoor, B.S. 2015. Choledocholithiasis: Diagnosis and Management. Tech Vasc Interv Radiol. 18(4):244-55.
2. Dyuzheva, T.G, Savitskaya, E.E, Kotovsky A.E, et al., 2012. Biodegradable materia, et ratio textus engineering in manu de bile ducts. In annalibus chirurgicam Hepatology, 17(1):94-99.
3. Kotovsky, A. E., Glebov K. G., 2008. Endoscopic transpapillary stenting of the bile ducts. The annals of surgical Hepatology, 13(1):66-71.
4. Katsikogianni, M, Spiliopoulou, I, Dowling D.P, Missirlis, Y.F. 2006. Adhesion of slime producing Staphylococcus epidermidis strains to PVC and diamond-like carbon/silver/fluorinated coatings. J. Mater. Sci. Mater. Med., 17(8):679-689.
5. Kulikovskii, V.F, Soloshenko, A.V, Iarosh, A.L et al., 2015. Use of meshendoprostheses with diamond-like carbon coating in abdominal hernias surgery. Khirurgiia (Mosk). (3):61-4.
6. Lebeaux, D., J. M. Ghigo, J. C. Lucet, 2014. Implanted medical device-related infections: pathophysiology and prevention. Rev. Prat., 64(5):620-625.
7. Parfenov, I.P, Iarosh, A.L, Soloshenko, A.V, Karpachev A.A, Sergeev O.S., 2011. Prediction of acute biliary pancreatitis. Khirurgiia (Mosk). (8):47-50.
8. Siiki, A., Helminen, M., Sand, J. et al., 2014. Covered self-expanding metal stents may be preferable to plastic stents in the treatment of chronic pancreatitis-related biliary strictures: a systematic review comparing 2 methods of stent therapy in benign biliary strictures. J. Clin. Gastroenterol., 48(7):635-643.
9. Xiu, Z. M., Zhang, Q. B., Puppala, H. L. et al., 2012. Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett, 12(8):4271-4275.
10. Wandling, M.W, Hungness, E.S, Pavey, E.S et al., 2016. Nationwide Assessment of Trends in Choledocholithiasis Management in the United States From 1998 to 2013. JAMA Surg. doi: 10.1001/jamasurg.2016.2059. [Epub ahead of print]