In vitro Study About Bacterial Adhesion to the Surface of Suture Materials Used in Oro- maxilo-facial Surgery

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Surgical sutures properly conducted will ensure maintaining the edges of the flaps until the wound healing process allows withstanding the functional stress. Among the possible complications of surgical sutures, we can find the wound suppuration, as a result of contamination with germs. In our in vitro study about the degree of bacterial adherence to different types of suture materials we chose four types of suture materials (three absorbable and one nonabsorbable) exposed to 4 bacterial species (Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa). The monofilament absorbable polydioxanone wire had the lowest bacterial adherence, followed by the multifilament nonabsorbable wire of silk and the two types of multifilament absorbable polyglycolic acid wires. Regarding the bacterial species tested, Enterococcus faecalis had the highest adhesion level, no matter what type of material or time of exposure has been used.

Key words: sutures wire, bacterial adhesion, polydioxanone, polyglycolic acid, silk

The suture of operative or accidental wounds represents the surgical act that realises the restoration of the structures continuity and aims to ensure quick and functional healing [1].

A right apposition of the wound edges in the maxillo-facial area is important for the patient's comfort, the hemostasis ensurance, the wound's size reduction, the prevention of bone loss and the avoidance of infection.

Suture materials should be strong, malleable, well tolerated and sterilizable. Their main classification criteria are: the origin, the ability to remain in the body and the thread type [2].

Depending on their origin, suture materials can be: natural (made of vegetable or animal polymers), artificial (obtained through chemical modification of natural polymers) or synthetic.

Regarding the remanence within the body, the suture threads can be absorbable or non-absorbable, and depending on the thread type, they can be monofilament or multifilament.

One of the possible postoperative complications is the wound suppuration, due to its contamination with aerobic germs. The most common germs are staphylococcus, streptococcus, E. coli, Proteus, Pseudomonas, Klebsiella. They mostly come from the digestive tract. To this complication also contribute certain local factors such as bleeding, hematoma from the wound, poor tissue vascularization, suture materials, incorrect drainage, long intervention, but also general factors such as: anemia, hypoproteinemia, diabetes, irradiation [3].

In the process of bacterial adhesion, a variety of mechanisms is involved, which is related to both the molecules from the bacterial wall and the various properties of the suture materials. To the bacterial adhesion contribute also: the Brownian motion, the hydrophobicity, the Van der Waals forces and the bacterial structures (fimbriae, flagella, pili, adhesins) [4].

Experimental part

The study was based on four types of suture materials (BIOSILK, PDOx, Bicril, Bicril Plus) and four bacterial species: Staphylococcus aureus (ATCC 43300), Enterococcus faecalis (ATCC 29212), Escherichia coli (ATCC 30040) and Pseudomonas aeruginosa (ATCC 27853).

BIOSILK are natural silk wires, coated with silicone, multifilament, nonabsorbable, black coloured, sterilized with ethylene oxide or gamma radiation.

PDOx is suture material based on polydioxanone, monofilament and absorbable. The wires are strands or violet, sterilized with ethylene oxide. The passage through the tissues is smooth and atraumatic, due to the monofilamental structure. The absorption is accomplished by a hydrolysis process within 120-210 days [5].

Bicril and Bicril Plus are multifilamental absorbable synthetic suture wires. The braided wires are coated with polycaprolactone and calcium stearate, are sterilized with ethylene oxide and are available in the form of strands or violet. Absorption is carried out by hydrolysis in 60-90 days and the multifilamental structure provides ease and safe achievment of surgical knots, while the coating turns the wire into a virtual monofilament that does not injure the tissues [6].

Bicril Plus is the antibacterial version, impregnated with chlorhexidine acetate.

Packages of suture wires were unsealed and were cut aseptically with sterile forceps and scissors, thirtysix 1 cm long fragments from each type of suture wire, in order to be used 9 fragments for each bacterial species studied. Fragments of suture wires were placed in sterile Petri plates.

For each bacterial species, inoculum McFarland 0.5 turbidity was prepared in glass tubes with metal plug, using a turbidimeter which analizes the density of bacteria in the solution. 1 (one) ml of each inoculum was then transferred with a sterile pipette in sterile Eppendorf tubes. Each fragment of the suture wires has been inserted in a

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Eppendorf tube with 1 mL inoculum and placed in contact with bacterial germs for 5, 60 and 120 s. Contacting bacteria with the suture wire was performed using a Comfort Eppendorf Thermomixer (Eppendorf AG, Hamburg, Germany), which realises a small rotation, set in this case at 300 rpm and a temperature of 23°C.

After each cycle, the fragment of the wire has been removed from the tube with bacterial inoculum, washed three times by immersion in a Erlenmeyer flask, filled with physiological saline, in order to remove non-adherent bacteria, and introduced in Eppendorf tubes, prepared in advance with 1 mL physiological saline for each bacterial species and exposure time. Each tube was then vortexed for 30s at 2100 rpm to detach the bacteria from the fragment of the suture wire.

From each vortexed tube, 50 mL of physiological saline have been pipetted using a automated pipette, which have then been applied to a simple agar medium and dispersed with a sterile loop over the entire surface of the medium (fig. 1a and b).

Culture media have been afterwards introduced into a incubator for 24 h, at a temperature of 37°C.

After the incubation period, the growth of bacterial colonies was shown on all culture media. The colonies were then counted with the Flash & Go colony counter (IUL SA Barcelona, Spain), which using the camera and auxiliary light sources, detects optically each colony on the culture medium.

The samples have been processed in the Microbiology Department of UMF Tg. Mures, using existing infrastructure and equipment in the department. Statistical has been analyzed through the ANOVA test, using SPSS software v22.0 Trial (IBM, Armonk, New York, USA).

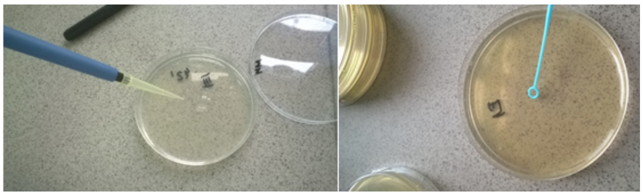

Fig. 1. a. Pipetting 50 mL of vortexed serum on the culture medium; b: The dispersion of the vortexed serum on the culture medium with a loop

Each test was repeated three times.

Results and discussions

For Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa, the ANOVA test showed that the suture material has interfered with the bacterial adhesion, statistically significant ($p < 0.01$) at each exposure level of the suture fragment with the bacterial inoculum (table 1, 3, 4).

On the other hand, bacterial adhesion of Enterococcus faecalis was not significantly affected ($P > 0.05$) by the suture material at the contact time of 60 s (table 2).

The PDOx material showed the lowest adherence for Staphylococcus aureus at all contact levels, followed by BIOSILK, Bicril Plus and Bicril, of which the staphylococcus has adherated the most (fig. 2).

In the case of Enterococcus faecalis, in the range of 5s Bicril Plus showed the minimum level of adhesion, for the period of 60s the suture material did not affect the statistical

Table 1
STAPHYLOCOCCUS AUREUS

	5 seconds	60 seconds	120 seconds
Biosilk	26.33 ± 2.51	102.0 ± 61.02	185.30 ± 51.02
PDOX	17.33 ± 1.52	32.33 ± 9.50	147.66 ± 37.28
Bicril	51.33 ± 3.51	295.0 ± 114.0	843.33 ± 123.50
Bicril Plus	15.33 ± 1.52	242.0 ± 48.04	212.0 ± 94.53
p value	p < 0.001	p < 0.01	p < 0.001

Each value is the average ([x20] ± standard deviation [x20]) of bacteria per ml.

Table 2
ENTEROCOCCUS FAECALIS

	5 seconds	60 seconds	120 seconds
Biosilk	1014.33 ± 25.50	1010.0 ± 297.0	517.0 ± 1.0
PDOX	716.0 ± 114.0	611.33 ± 177.50	510.0 ± 176.55
Bicril	1144.0 ± 218.0	982.33 ± 29.50	1057.33 ± 287.50
Bicril Plus	315.33 ± 45.50	787.33 ± 6.50	1043.0 ± 168.0
p value	p < 0.001	p > 0.05	p < 0.01

Each value is the average ([x20] ± standard deviation [x20]) of bacteria per ml.

Table 3
ESCHERICHIA COLI

	5 seconds	60 seconds	120 seconds
Biosilk	44.33 ± 3.51	73.33 ± 2.51	46.0 ± 4.0
PDOX	9.33 ± 4.72	31.66 ± 12.74	14.0 ± 7
Bicril	32.33 ± 4.50	110.66 ± 4.04	125.0 ± 35.0
Bicril Plus	83.0 ± 8.0	61.33 ± 24.50	65.33 ± 21.50
p value	p < 0.001	p < 0.01	p < 0.001

Each value is the average ([x20] ± standard deviation [x20]) of bacteria per ml.

Table 4
PSEUDOMONAS AERUGINOSA

	5 seconds	60 seconds	120 seconds
Biosilk	351.0 ± 51.11	598.33 ± 11.50	601.66 ±112.03
PDOX	195.0 ± 1.0	195.33 ± 18.82	462.0 ± 31.0
Bicril	548.33 ± 27.50	699.0 ± 19.0	980.0 ± 120.0
Bicril Plus	320.66 ± 74.46	568.33 ± 18.50	853.33 ± 234.66
p value	p < 0.001	p < 0.001	p < 0.01

Each value is the average ([x20] ± standard deviation [x20]) of bacteria per ml.

bacterial adherence (p> 0.05), and for 120 s PDOx and BIOSILK presented the lowest adhesion (fig. 2).

The polydioxanone suture material PDOx had the lowest adherence for both Escherichia coli and Pseudomonas aeruginosa at all time intervals compared to other materials, followed by BIOSILK, Bicril Plus and Bicril, the last having the highest bacterial adherence (fig. 4, 5).

Among the bacterial species, Enterococcus faecalis (fig. 6) had the highest adhesion to suture materials, followed by Pseudomonas aeruginosa (fig.7), Staphylococcus aureus (fig. 8) and Escherichia coli (fig. 9) which presented the lowest level of bacterial adhesion (fig. 10).

Increasing the exposure time of the bacterial inoculum with fragments of the suture wires from 5 to 120 s, has conducted to a significant increase (p <0.01) of the

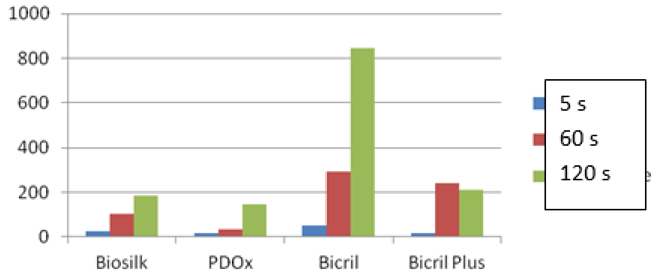

Fig. 2. Bacterial Adhesion for Staphylococcus aureus

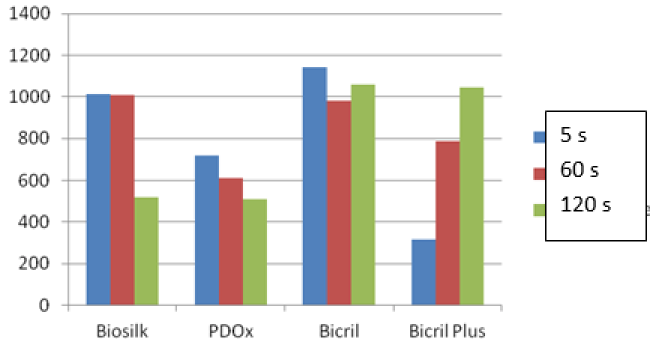

Fig. 3. Bacterial Adhesion for Enterococcus faecalis

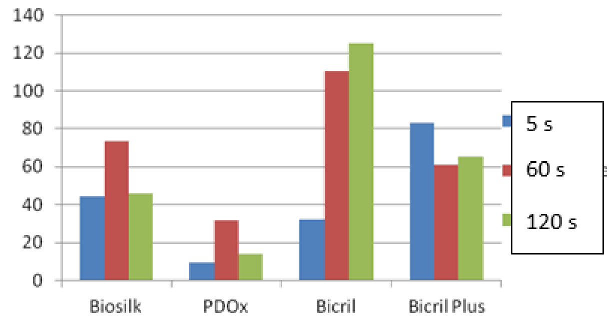

Fig. 4. Bacterial Adhesion for Escherichia coli

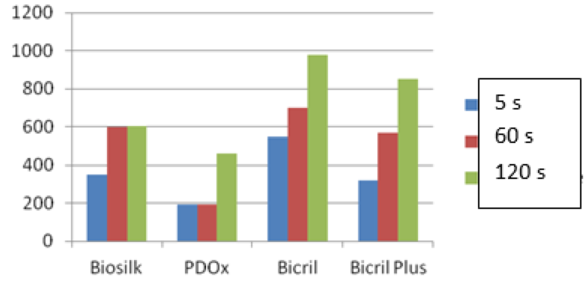

Fig. 5. Bacterial Adhesion for Pseudomonas aeruginosa

bacterial adhesion for the species Staphylococcus aureus, Pseudonomas aeruginosa and Escherichia coli, on the other hand Enterococcus faecalis germs adhesion was not influenced by the exposure time (fig. 11).

Bacterial adhesion to suture wires is a subject studied in the literature for over 30 years, the structural and chemical composition of suture materials being correlated with bacterial adhesion and tissue reaction. From these studies, it is clear that any material, absorbable or non-absorbable, mono- or multifilament, is susceptible to bacterial adhesion and has the ability to induce a immune inflammation response in the surrounding tissues. [7]

The oral cavity is an environment that hosts a diverse bacterial community, abundant and highly complex which actually gives it a high risk of infection of the sutured wounds. Suture wires in the oral cavity are located in a salivary medium, containing approximately 7.5 x 10^8/mL bacterial micro-organisms and will most likely to be

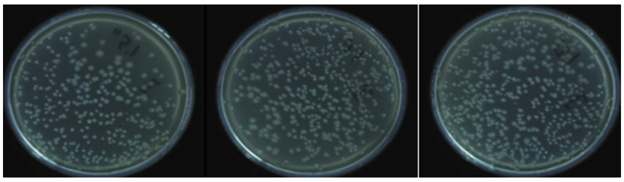

Fig. 6. Colonies of Enterococcus faecalis after 24 h at the three intervals of contact with the suture thread (5, 60 and 120 s).

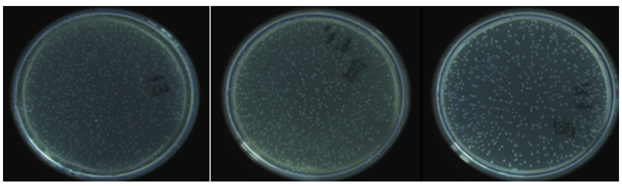

Fig. 7. Colonies of Pseudonomas aeruginosa after 24 h at the three intervals of contact with the suture thread (5, 60 and 120 s).

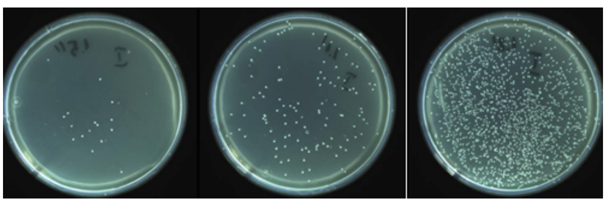

Fig. 8. Colonies of Staphylococcus aureus after 24 h at the three intervals of contact with the suture thread (5, 60 and 120 s).

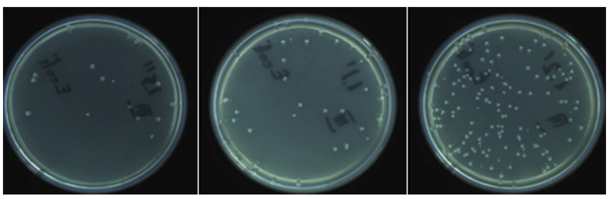

Fig. 9. Colonies of Escherichia coli after 24 h at the three intervals of contact with the suture thread (5, 60 and 120 s).

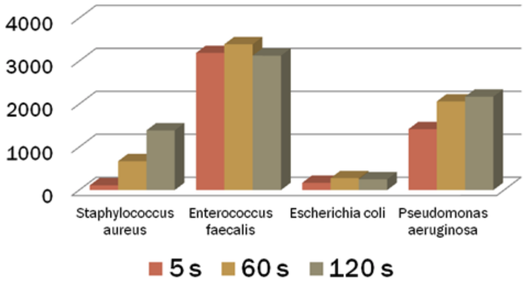

Fig. 10. The total number of colonies of each bacterial species

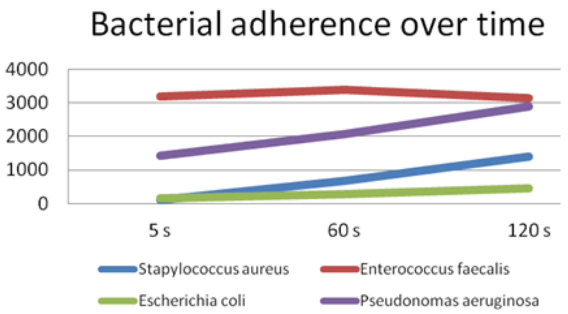

Fig. 11. Total number of colonies depending on the time of exposure to the bacterial inoculum of the suture material

covered by the biofilm which enhances bacterial adhesion to the suture wires. Thus it would be preferable to use a suture material that can reduce the colonization level of bacterial germs [7, 8].

The biofilm is a polymer matrix made of cells that adhere to surfaces and to each other, constituting a reservoir for bacteria where they are *hidden* from the immune system of the host and antibiotics. Complete elimination of the biofilm may be impossible [9].

Suture wires placed in the region of the neck and mouth require a high tensile strength that leads to the preferential use of multifilament wires and not monofilament ones [8].

The results of this in vitro study revealed significant differences of bacterial adherence, depending on the suture material tested. Overall, PDOx showed the lowest level of bacterial adhesion, due to its monofilamental structure, with smooth surface. These qualities confer a minimum capillarity, reducing the risk of infection, the tissue reaction, but because of its structure, handling this type of thread is difficult and knots are not safe, having a low tensile strength.

The polyglycolic acid material Bicril had the highest bacterial adherence and Bicril Plus, its antimicrobial version, showed only a slight reduction of adhesion. Being multifilamental suture wires, they have a high capillarity that allows the colonization and permeability by bacterial germs, as well as the increasing of infection risk. On the other hand, it has the advantage that it can be easily maneuvered and that it ensures the safety of the nodes. The absorbable material may induce some inflammatory immune responses, which lead to the infection of sutured wounds [10].

Therefore, the data presented in this study indicate a major differences between the types of suture materials, monofilamental or multifilamental, regarding their bacterial adhesion.

Both extra-oral and intra-oral suture wires are considered as foreign by the human body and may increase the infection risk of the sutured wound. In the contaminated tissues, it is indicated to use monofilamental absorbable sutures, in order not to turn contaminated plagues into infected ones. [11]

Among the analyzed bacterial species in this in vitro study, Enterococcus faecalis had the highest level of bacterial adhesion on all suture materials, not influenced by the exposure time of the inoculum with the surface of the tested wire. This can be explained by the fact that some strains have the ability to develop pili, which could enable a faster formation of the biofilm and in an increased amount. [12] The other species had a directly proportional adherence to the exposure time.

The results of this in vitro study are consistent with those presented by Matalon et al. (2013) which showed that the expousure time, the bacterial species and the suture type have significantly influenced ($p < 0.001$) the statistics of bacterial adhesion for Staphylococcus aureus and Pseudomonas aeruginosa. [13] The high level of bacterial adherence to the suture material in polyglycolic acid was also demonstrated by Masini et al. (2011) [48].

It is necessary to confirm the results of this in vitro study by continuing the in vivo research of the bacterial adherence level to the biomaterials structures and its mechanism to induce infection within the tissue it is implanted in.

Conclusions

The polydioxanone absorbable monofilament wire of PDOx had the lowest bacterial adherence level, followed by the multifilament nonabsorbable natural silk wire, BIOSILK and finally the two types of polyglycolic acid absorbable multifilament wires, Bicril and Bicril Plus.

From all the bacterial species tested, Enterococcus faecalis had the highest adhesion level, regardless of the material type or time of exposure.

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