



## Dynamics of Changes in Resistance of Surgical Sutures in Environment of Sterile and Contaminated Bile – *In Vitro* Research

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### Abstract

**Mini-abstract:** The study presents results of the examination of resistance of surgical sutures (Polidioxanone, Poliglecaprone and Poliglactin 910 as well as their analogues coated with antibacterial triclosan) in sterile and contaminated bile. The research has been performed in *in vitro* conditions.

**Structured abstract:** The choice of appropriate surgical suture during operation is of much significance. Currently, there are no objective studies regarding the resistance of commonly used sutures in biliary tract surgery. The fact leads one to conduct the research concerning resistance of the sutures (Polidioxanone, Poliglecaprone, Poliglactin 910 and their analogues coated with antibacterial triclosan) in the environment of sterile and contaminated bile. The study was performed in *in vitro* conditions for 28 days. The study indicated that sutures made of polidioxanone had the most supreme qualities during entire experiment.

**Keywords:** Surgical sutures; Resistance; Bile

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### Introduction

The choice of appropriate surgical suture for the type of anastomosis is one of the conditions necessary for its success. Incorrectly chosen sutures may cause multiple complications that threaten the life of a patient. Surgical sutures are subject to extreme effects in human organism. Conditions in human organism vary and are dependent on characteristics of the organ such as low or high pH, action of the enzymes, bacteria and mechanical forces. In literature [1-6] one may encounter information concerning post-operative complications associated with premature dehiscence of the sutures in anastomoses exposed to bile activity (biliary-intestinal anastomoses, biliaro-biliary) i.e., Pancreatoduodenectomy and/or palliative surgical bypass anastomoses. Bile affects surgical sutures by accelerating their degradation [7]. At present, in order to prevent infection, sutures coated with antibacterial substance are used. So far, there has been no research conducted concerning resistance of the sutures coated with triclosan in the environment of sterile and infected bile. However, there are studies assessing resistance of sutures in sterile bile regarding sutures without antibacterial coating.

### Methods

The aim of the study was to determine resistance of the sutures coated with antibacterial agent (PDS Plus, Vicryl Plus, Monocryl Plus) and with antibacterial coating – Polydioxanone (PDS, Vicryl, Monocryl). The aforementioned sutures were of 3-0 size. The sutures were immersed in 3 following environments: Sterile bile, contaminated bile. Physiological saline was used as a control environment (0.9% NaCl).

### Biological material

The bile was obtained from a patient undergoing pancreatic tumor treatment. Bile was

collected from T drain (Kehr) inserted into common bile duct during the procedure. The material was collected from 1 patient fourfold (in 4 following post-operative days). Biological material was distributed into sterile criotubes (Corning® Internal Threaded Polypropylene Cryogenic Vial, USA) of 5 ml volume. Biological material was distributed in sterile conditions. Sterility of the material was confirmed bacteriologically in each probe prior to freezing (microscopic examination and microbiological culture collected after four days from each sample). Microscopic examination confirming the presence of the bacteria excluded the material from the freezing process. The culture obtained after four days confirmed the sterility of the frozen material. If the culture was positive, the material was excluded as well. Prior to freezing, pH of the bile was measured with means of laboratory pH meter (Piccolo HI 98111, Hanna Instruments, USA). Biological material in crioprobe was frozen and contained in laboratory freezer (Revco™ High-Performance Lab Freezers ULT430A, Thermo Fisher Scientific, USA) in temperature  $-20^{\circ}\text{C}$  [8].

### Bacteriological examination

Two methods of bacteriological examination were performed. Microscopic examination of the material (Kern OBN - 14, Kern Optics, Germany). If in the high power field presence of the bacteria in bile was noted the probe was excluded from the test. Additionally, the microbiological culture was performed. Growth media for the bacteria, fungi and mold were used. The media used in the study: MacConkey, Kligler, Clauberg, Chapman, Sabouraud. If the results of both microbiological and microscopic examination were negative, the material was considered sterile. Similar means of bacteriological examination were introduced after defrosting the material from criotubes. Attenuated material was obtained by contamination of bile with 1) *Echerichia coli*, 2) *Klebsiella spp.* and 3) *Enterococcus faecalis* (bacteriae most frequently occurring in pancreatic and biliary tract infections) [9-13]. For the purpose of contamination one prepared three bacterial suspensions (consisting of sterile saline and particular bacterial strain), which were examined according to MacFarland 0.5 standard with turbidity meter (MicroScan TurbidityMeter, Siemens AG, Germany,) [14]. Following the turbidimetric examination, using pipette (Eppendorf Xplorer®, Eppendorf, Germany) an amount of 10  $\mu\text{L}$  of each suspension was collected and added to probes containing sterile bile. Part of the study was conducted in a certified analytical laboratory. The sutures were immersed in biological material and incubated in sterile crioprobe in a laboratory incubator.

### Course of experiment

Figure 1 presents course of the experiment. To the tubes numbered 1, 2, 3, 4, 5, 6 with the defrosted bile one inserted respectively 24 pieces of PDS, Monocryl, Vicryl, PDS Plus, Monocryl Plus, Vicryl Plus. The probes with the sutures were incubated in a laboratory incubator (Hanna® COD Test Tube Heater (HI839800-01), Hanna Instruments, USA) in  $+37^{\circ}\text{C}$  temperature [8,15]. Resistance measurements of the sutures were performed on tensile testing machine INSTRON 4469 (Instron®, USA). The resistance examination was performed at 0-7-14-21-28 day of the immersion of the sutures. Day "0" is the date of the measurement of a suture collected directly from a package (base, initial state). At specific dates, one removed from the test tube 6 pieces of the sutures and examined their resistance. Bile was exchanged every 24 h, daily. After bile defrosting bacteriological examination of liquid in each tube was performed according to a schedule described previously.

### Statistical methods

Database of the clinical material was created under licensed version of Excel spreadsheet v. 2003 (Microsoft). The data was implemented into Statistica package v. 7.1 (Statsoft) and statistical software MEDCALC v. 11.3.1 (MedCalc Software). At the first stage one has to estimate the basic characteristics of descriptive statistics of the resistance of examined sutures including: Arithmetic mean, median, maximum and minimal value, quartile 25% (lower), quartile 75% (upper), standard deviation, standard error of the mean SEM, kurtosis and skewness. In the statistical evaluation one accepted the significance level of  $p(a)<0.05$ . Tests used: Shapiro-Wilk test to verify the hypothesis of normal distribution of the characteristics, Levene test to verify hypothesis of homogeneity of variance, ANOVA one-way test to verify the hypothesis of equality of means among the groups, post-hoc test reasonably significant difference, t Student test for the two means, Fisher homogeneity variance test, of the variance is not homogeneous: Satterthwaite test.

### Results

Dependence between resistance and environment as well as exposure time - sutures without antibacterial coating (Monocryl, PDS, Vicryl)

#### Monocryl

Analyzing the results of Monocryl sutures resistance studies, statistically significant differences at day 21 were noticed, where Rm level of Monocryl sutures in environment of sterile bile is higher than in environment of contaminated bile ( $p=0.0153$ ). It is a confirmation that surgical sutures suffer greater destruction in contaminated environment than in sterile one. It is worth noticing that for Monocryl sutures the last possible measurement of resistance was day 21 of the study (at day 28 sutures were destroyed completely).

#### PDS

One noticed that for the variables there are statistically important differences at day 21 of the exposition, level of resistance for PDS sutures in environment of sterile bile is higher than in contaminated one ( $p=0.0172$ ). Simultaneously, one proven the influence of contaminated environment on resistance. However, PDS sutures lasted until day 28 of the examination. If one analyzes levels of resistance presented in the Figure 2, it can be noticed that PDS resistance decreases statistically insignificantly in comparison to initial values. PDS resistance is practically constant.

#### Vicryl

There are statistically significant differences at 7, 14, 21 day of exposition in Rm level for Vicryl sutures in sterile bile. Resistance (Rm) is higher in sterile environment than in contaminated one ( $p<0.05$ ). At that stage in the study one has proven essential influence of the environment on decrease of Vicryl sutures resistance. Day 21 was the last where resistance measurements could be obtained (Figure 3).

Dependence between resistance and environment as well as exposure time - sutures with antibacterial coating (Monocryl Plus, PDS Plus, Vicryl Plus).

#### Monocryl plus

There are statistically significant differences at 7, 14 days of exposition in Rm level for Monocryl Plus sutures. Rm in environment of sterile bile is higher than in contaminated one ( $p<0.05$ ). One

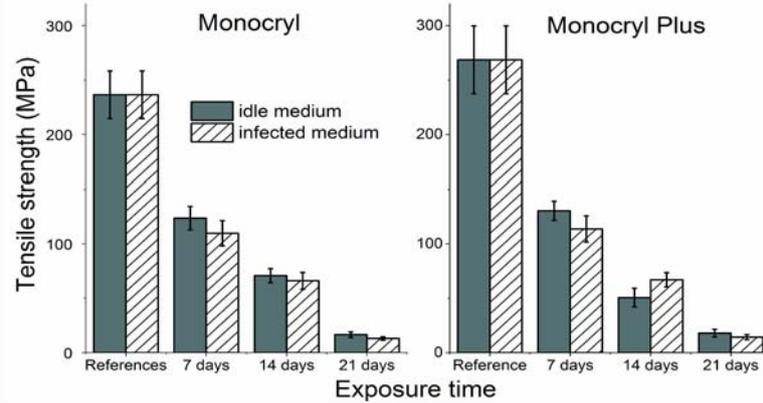


Figure 1: Resistance measurement results Rm (MPa) of Monocryl and Monocryl Plus sutures in infected and sterile bile dependant on the exposure time.

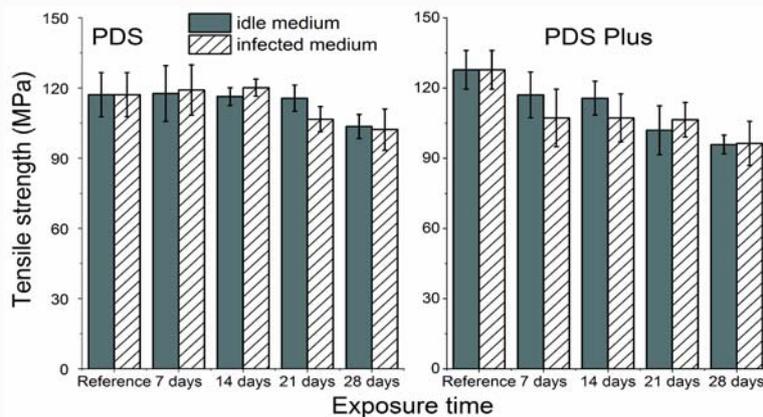


Figure 2: Resistance measurement results Rm (MPa) of PDS and PDS Plus sutures in infected and sterile bile dependant on the exposure time.

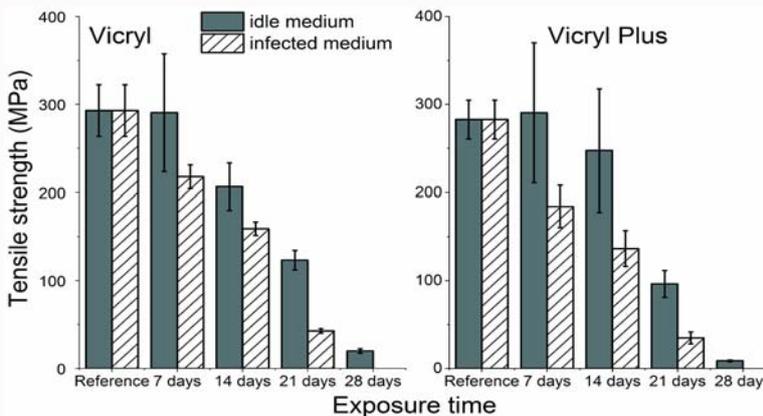


Figure 3: Resistance measurements results (Rm) of Vicryl and Vicryl Plus sutures in infected and sterile bile dependant on the exposure time.

concluded that in the case of Monocryl Plus antibacterial coating had influence on the resistance of suture in environment of contaminated bile. At day 21, resistance in terms of exposure is comparable both in sterile and contaminated environment. Day 21 was the day of a final measurement (at day 28 sutures were entirely destroyed).

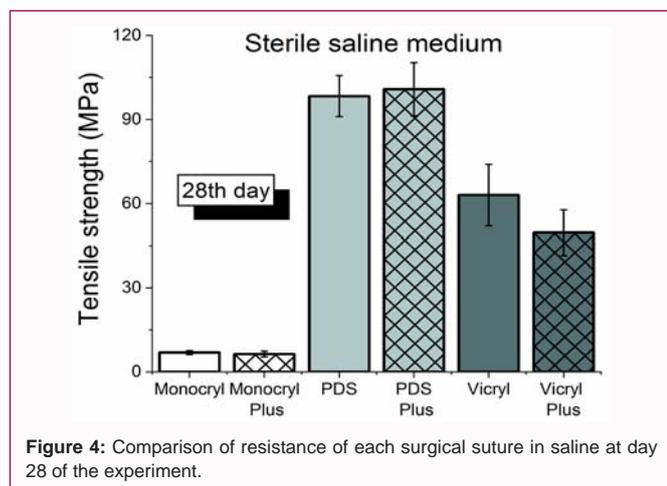
**PDS plus**

There are no statistically significant differences in resistance level of PDS Plus sutures in sterile and contaminated bile environment. Thus, one may assume that antibacterial coating of PDS sutures has no significant influence on the resistance of the sutures in both sterile

and contaminated environment.

**Vicryl plus**

There are statistically significant differences at 7, 14, 21 days of exposition. Rm level for Vicryl plus sutures in environment of sterile bile is higher than one in contaminated environment at dates given ( $p < 0.05$ ). One states that antibacterial coating of Vicryl plus sutures does not provide greater resistance of the sutures in infected environment. In the study saline was a control sample. Therefore, one was able to determine the influence of the environments. As it is presented in the Figure 4. Each of the sutures are characterized by



**Figure 4:** Comparison of resistance of each surgical suture in saline at day 28 of the experiment.

determinable resistance at day 28 of the examination. Indisputable fact is that environment of sterile and contaminated bile influences disintegration of the sutures and the result is decrease in their resistance.

## Discussion

In the study PDS and PDS Plus sutures presented the highest resistance. Muftuoglu as well noticed that PDS sutures were characterized by the lowest decrease in resistance value. The conclusion was that PDS was the most resistant type of the sutures compared at 1 to 7 day of the experiment (in comparison to Vicryl, silk sutures, catgut chrome and propylene sutures) Tian suggested that in pancreatic and biliary tract surgery one should use sutures which decompose slowly and without sudden changes in resistance-Dexon and Maxon were compared [7]. In the following study PDS suture fall under the criteria. Freudenberg et al. indicated a significant decrease in resistance of Vicryl sutures in pancreatic juice, whereas PDS suture was highly stable in the conditions [8]. Similar results were the outcome of the following study. Chung proved influence of the bacteria directly on polymers of which the sutures were made of due to secretion of lytic enzymes or changes in pH of the environment [16]. It may serve as an explanation of the influence of the bacteria on resistance of surgical sutures. In following study, one has proven the impact of contaminated environment on degradation of sutures and decrease in resistance of the sutures. PDS and PDS Plus sutures had the highest resistance. Muftuoglu noticed as well that PDS sutures had the lowest decrease in resistance measurement values [15]. Chung noticed that presence of *E. coli* bacteria in *in vitro* conditions in bodily fluids influences the sutures and results in decrease in their resistance. The results of following study also proved influence of contaminated environment on degradation of the sutures and decrease in resistance. It may explain the influence of bacteria presence on resistance of surgical sutures. While analyzing resistance of Monocryl and Vicryl sutures one notices that it differs significantly in environment of contaminated and sterile bile. Monocryl is a monofilament whereas Vicryl is braided therefore a suspicion that Triclosan antibacterial coating is correlated with spatial structure of sutures. Nevertheless, further examination of microstructure of sutures is needed. The following study provides answer to a question which sutures to choose in anastomosis exposed to action of bile. If one suspects that healing will be demanding and prolonged (may result from i.e., the patient's different comorbidities) it is advisable to select a suture, which ability to sustain tension (in *in*

*vitro* conditions the last determined resistance) is the most prolonged. The results of the authors own research indicate that the sutures made of polidioxanone are characterized by already mentioned proprieties. The study revealed that antibacterial coating poorly influences resistance of sutures in examined environments. Advantage of sutures with antibacterial coating has not been indicated. Taking into consideration the results of the study, choice of suitable suture for anastomosis may become easier and number of complications resulting from inappropriate decision may decrease.

## Conclusion

Surgical sutures examined in the study vary in resistance within the group. Presence of bacteria in environment (contaminated environment) influences dynamics of resistance of sutures resulting in its decrease. Triclosane antibacterial coating has no influence on dynamics of change in resistance in contaminated environment. Resistance of surgical sutures is dependent on exposure time in environment and statistically significant decrease of resistance in each environment is noticeable. Polidioxanone (PDS and PDS Plus) is material of the highest resistance and most extended degradation period.

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