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# Journal of Life Science and Biomedicine



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# Journal of Life Science and Biomedicine

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## Volume 9 (1); January 25, 2019

### Research Paper

#### Features of the psychoemotional condition of women with induced pregnancy.

Asatova MM, Saidazova ShSh, and Voitova GA.  
*J. Life Sci. Biomed.*, 9(1): 01-04, 2019;  
 pii:S225199391900001-9

#### Abstract

The objective of the present study was to evaluate special features of the psychological health of women with induced pregnancy and its relationship with nausea and vomiting. The Spielberger scale score results of 270 women with nausea and vomiting during of induced-pregnancy (NVIP) has been studied. The mean age of the women was  $25 \pm 4.2$  years. Results showed that, 31 (11.5%), 118 (43.7%) and 121 (44.8%) of pregnant women with NVIP had low, moderate and high degree of anxiety, respectively. Most of the surveyed women with NVIP (72.2%) experienced a high level of situational anxiety. For moderate and low degrees of state anxiety, the approximately equal frequency was registered, 14.1% (n=38) and 13.7% (n = 37), respectively. The state of tension and regretful from the current situation was noted by 175 (64.8%), anxiety and nervousness were registered in 158 (58.5%) and 207 (76.7%) cases, respectively. 147 (54.4%) and 187 (69.3%) women, respectively, noted their excitement for possible failures and concern. 72.2% of women with induced pregnancy have state anxiety as a result of the emotional reaction to NVIP, which indicates the need for counseling by psychologists.

**Keywords:** Induced pregnancy, First trimester, Nausea and vomiting, Trait and state anxiety, Spielberger scale

[Full text-[PDF](#)]



### Research Paper

#### Impact of school meals' type and time on children's food consumption, physical and behavioral activities.

Yousefi M and Yousefi Z.  
*J. Life Sci. Biomed.*, 9(1): 05-09, 2019;  
 pii:S225199391900002-9

#### Abstract

Today, proper nutrition is one of the useful tools for the healthiness and sustainability of people's diet and especially student performance and behavior in school. Existing nutrient standards for school meals are always important when packed foods or lunches brought from home. The aim of the present study was to determine the effects of school meals type and time on behavioral response, physical activity and the body mass index (BMI) in elementary students of Razan city, Iran. Elementary school principals (N = 16) and total of 234 students selected from 5 schools completed a survey on the school food and physical and behavioral activities environment. Students were weighed and measured for their body mass index (BMI) that calculated using a standard protocol and also send a BMI report card to their parents. Results of this study showed that effect of type of time regulation in school nutritional program could significantly improve behavioral response, and especially physical activity and BMI and it can be considered as an applicable strategy to the implementation of such programs on the health status of students.

**Keywords:** Elementary students, Body mass index, Nutritional behavior, Physical activity

[Full text-[PDF](#)]



### Research Paper

#### Analysis of heavy metal content of Cu, Pb, Hg and dissolved Sn in coastal of Banyuwangi district, Indonesia.

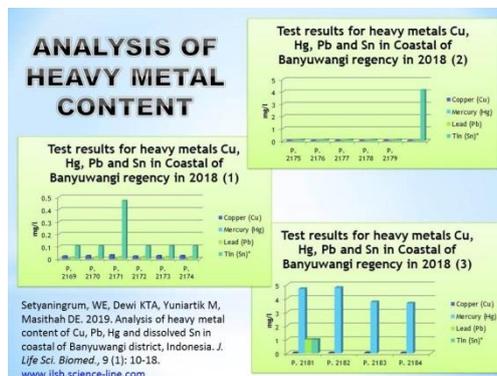
Setyaningrum, WE, Dewi KTA, Yuniartik M, Masithah DE.  
*J. Life Sci. Biomed.*, 9(1): 10-18, 2019; pii:S225199391900003-9

## Abstract

Banyuwangi Regency has the longest coast in East Java of Indonesia with sandy beaches and corals and there are various types of coastal and marine resources that can be utilized both in terms of economics and environment. But in the current era of industrialization, coastal areas in Banyuwangi have become a top priority for industrial development, agribusiness, agro-industry, housing, transportation, ports and tourism. The purpose of this study was to analyze the content of copper (Cu), lead (Pb), mercury (Hg), and tin (Sn) and the effect of water quality on the heavy metal content in the coast of Banyuwangi Regency. The method in this study uses descriptive. Data taken along the coast of Banyuwangi Regency include water quality (alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature and salinity), copper (Cu), lead (Pb), mercury (Hg), and tin (Sn). Data analysis using multiple linear regression analysis, followed by F test and t-test. The results showed that there was an influence between the quality of the water on the value of heavy metal of copper (Cu), and the value of R-Square 0.681 which means that it has an influence proportion on the value of copper (Cu) of 68.1%. Likewise, for the quality of water for tin (Sn), there is an influence with the value of R-Square of 0.700, which means that the effect is as high as 70%. While the quality of the waters against Lead, heavy metal (Pb) and mercury (Hg) has no significant effect. Based on the results of the study, Banyuwangi district government needs to take serious actions in controlling heavy metal pollution through the implementation of law No. 23 of 1997 concerning to environmental management, and the application of environmental quality standards more strictly.

**Keywords:** Banyuwangi coastal, Copper (Cu), Heavy metals, Lead (Pb), Mercury (Hg), Tin (Sn), Water quality

[Full text-[PDF](#)]



## Research Paper

### New hemostatic preparation made of the cellulose derivatives.

Sadykov RA, Ismailov BA, and Valerevna KO.  
*J. Life Sci. Biomed.*, 9(1): 19-25, 2019;  
pii:S225199391900004-9

#### Abstract

**Aim.** The aim of this study was to investigate the indexes the indexes of biocompatible pellicle hemostatic coverage in vitro and in vivo conditions.

**Methods.** Samples of pellicle hemostatic coverage on the basis of the cellulose derivatives were used in researches. Breaking strength, estimation of the implant's structure and adhesion power were evaluated according to the Ts 05957837-28:2014 instructions and documents of the National certification system of the Republic of Uzbekistan and with the using the apparatus "Zwick" (Germany) and atomic-power microscope of Agilent technologies (USA). Hemostatic activity of the coverage on the basis of the cellulose derivatives was estimated by the Lee and White test for the blood coagulation time. For in vivo research, 30 mature rats were required. Operations were performed under inhalation anesthesia, and the wound of liver was formed. Both macroscopic and microscopic studies had been undertaken. Morphological changes were studied in terms of 3 and 12 hours and then on the 1st, 3d, 7th, 14th and 30th day after an operation.

**Results.** An adhesion power of the pellicle coverage on the basis of the cellulose derivatives was  $7.3 \pm 0.2$  N/cm<sup>2</sup>, breaking strength was  $390 \pm 4.8$  kGf/cm<sup>2</sup>. In presence of polymer, a coagulation time on Lee and White test was shortened by as many as 2.1 times in relation to control that made up  $2.4 \pm 0.6$  min. In in vivo conditions hemostasis started during 3-5 sec. A weak inflammatory reaction of tissue was histologically determined. Further observations over dogs showed that an hour after an operation, an implant had been preserved on the surface of liver as a white pellicle and had not been separated from the wound surface. Bleeding signs were not marked. An abdominal cavity remained intact. **Conclusion.** Rapid enough biodegradation of polymer along with the unexpressed inflammatory reaction allows preventing the infecting related to the presence of foreign body. The rapid forming of fibrotic tissue in a zone of lesion makes it possible to obtain a durable hemostasis. A poorly expressed reaction was also marked from the side of peritoneum and surrounding organs. **Recommendations.** The oxidized regenerated cellulose can result in an intensive inflammation of the surrounding tissues because of the low level of pH that had not happened in the present research due to the selected correlation of ingredients of hemostatic pellicle.

**Keywords:** Hemostasis, Morphology, Carboxymethylcellulose, Oxidized Cellulose, Hemostatic Substance.

[Full text-[PDF](#)]

## Review

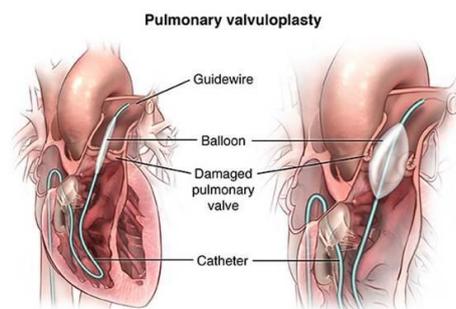
### Transluminal balloon valvotomy in the surgical treatment of mitral stenosis in pregnant women: a review.

Zufarov MM and Abdullaeva MA.  
*J. Life Sci. Biomed.*, 9(1): 26-33, 2019; pii:S225199391900005-9

## Abstract

**Aim.** The aim of this study was to investigate current strategies in treatment of pregnant women suffering with mitral stenosis. Mitral stenosis is an obstruction of the pathway of the left ventricle flow at the mitral valve (MV) level as a result of the structural deformation of the MV apparatus, which prevents the necessary opening of the MV during the diastolic filling of the left ventricle. The most frequent lesion of valves in women with rheumatic heart disease (RHD) is MV. It remains the most common acquired valvular lesion in pregnant women and is one of the main causes of maternal death from cardiovascular diseases. According to the literature mitral stenosis (MS) is found in 75–90% of pregnant women with acquired heart defects. In addition, the incidence of fetal morbidity is positively correlated with the severity of MS: it increases from 14% in pregnant women with mild MS to 28% in people with moderate MS and 33% in women with severe mitral stenosis. The course of MS in pregnant women depends on the clinical manifestations, the degree of severity of heart failure, and the degree of rheumatic fever activity. **Conclusion.** The advantages of minimally invasive techniques during pregnancy are theoretically undeniable. The choice of the optimal method of delivery, the assessment of the fetal state of the fetus and the methods for its correction are also fundamental. **Keywords:** Mitral stenosis, Left ventricle, Pregnancy, Women

[Full text-[PDF](#)]



To cite this paper: Zufarov MM and Abdulleeva MA. 2019. Transluminal balloon valvotomy in the surgical treatment of mitral stenosis in pregnant women: a review. *J. Life Sci. Biomed.* 9(1): 26-33; [www.jlsb.science-line.com](http://www.jlsb.science-line.com)

The advantages of minimally invasive techniques during pregnancy are theoretically undeniable. The choice of the optimal method of delivery, the assessment of the fetal state of the fetus and the methods for its correction are also fundamental.

## Research Paper

### Comparative efficacy of the hemostatic implant made of the cellulose derivatives on the model of parenchymatous hemorrhage from liver.

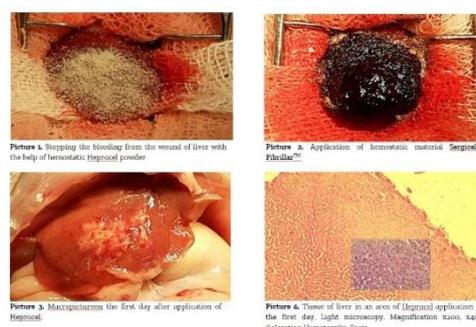
Ismailov BA, Sadykov RA, and Mirzaahmedov BM.  
*J. Life Sci. Biomed.*, 9(1): 34-41, 2019;  
pii:S225199391900006-9

#### Abstract

**Aim.** The aim was to study the comparative efficiency of the hemostatic implant made of the cellulose derivatives on a model of the parenchymatous hemorrhage from a liver. **Methods.** Experimental studies on the biocompatibility's evaluation were conducted in accordance with the Russian national standard ISO 10993-6-2011. Operations have been performed under the general anaesthetizing with the modeling the parenchymatous hemorrhage from the wound of liver. A total of 72 white mature outbred rats of both sexes weighing  $196.5 \pm 2.8$  g were used from which 36 ones made up group of comparison using the application hemostatic material, Sergicel® Fibrillar™. In the basic group of rodents (36) powder Heparin in equal amounts by weight of 30 mg was applied on a wound. **Results.** From the results it is possible to come to a conclusion that the hemostatic Heparin implant causes on the first day morphological reaction of liver as an inflammation and a spread of the connecting tissue, but these processes calm down quickly. An inflammatory reaction was less expressed than the control group. To the 30th day in the basic group after application of Heparin biodecomposition of hemostatic implant was being marked, there were regenerator processes in the liver's parenchima especially in the zone of lesion that testifies to renewal of liver's tissue, while in a comparison group an active degradation of the application hemostatic material began on the 30th day and an expressed adhesion process in an abdominal cavity took place. **Conclusion.** Hemostatic powder closely adjoins the liver's tissue, stops bleeding, cases of relapse of bleeding were not marked. Histological researches conducted in the dynamics of the healing showed that the wounds of liver educed that Heparin did not cause the expressed inflammatory reaction, the zone of lesion did not exceed 150  $\mu$ m, and the biodecomposition started after 14 days.

**Keywords:** Hemostasis, Parenchymatous Hemorrhage, Liver, Cellulose's derivatives, Experimental surgery, Hemostatic implant, Morphology of liver.

[Full text-[PDF](#)]



To cite this paper: Ismailov BA, Sadykov RA, and Mirzaahmedov BM. 2019. Comparative efficacy of the hemostatic implant made of the cellulose derivatives on the model of parenchymatous hemorrhage from liver. *J. Life Sci. Biomed.* 9(1): 34-41; [www.jlsb.science-line.com](http://www.jlsb.science-line.com)

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# Features of the psychoemotional condition of women with induced pregnancy

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## ABSTRACT

The objective of the present study was to evaluate special features of the psychological health of women with induced pregnancy and its relationship with nausea and vomiting. The Spielberger scale score results of 270 women with nausea and vomiting during of induced-pregnancy (NVIP) has been studied. The mean age of the women was 25±4.2 years. Results showed that, 31 (11.5%), 118 (43.7%) and 121 (44.8%) of pregnant women with NVIP had low, moderate and high degree of anxiety, respectively. Most of the surveyed women with NVIP (72.2%) experienced a high level of situational anxiety. For moderate and low degrees of state anxiety, the approximately equal frequency was registered, 14.1% (n=38) and 13.7% (n = 37), respectively. The state of tension and regretful from the current situation was noted by 175 (64.8%), anxiety and nervousness were registered in 158 (58.5%) and 207 (76.7%) cases, respectively. 147 (54.4%) and 187 (69.3%) women, respectively, noted their excitement for possible failures and concern. 72.2% of women with induced pregnancy have state anxiety as a result of the emotional reaction to NVIP, which indicates the need for counseling by psychologists.

## Original Article

PII: S225199391900001-9

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## Keywords

Induced Pregnancy,  
First Trimester,  
Nausea and Vomiting,  
Trait and State Anxiety,  
Spielberger Scale

## INTRODUCTION

It is known that the ratio of indicators of a woman's mental health (stress resistance, trait anxiety, emotionality and emotional reactivity) form a single system during pregnancy and reflects the general psycho-emotional condition of a woman [1, 2].

Most often, changes in the psycho-emotional background during pregnancy lead to the development of depressive and anxiety disorders. Changes in the psycho-emotional state of women who have long suffered from infertility are of particular relevance.

Symptoms of anxiety during pregnancy can relate to several types of anxiety, such as general anxiety, anxiety disorders and anxiety related to pregnancy, anxiety characterized by specific fears and anxieties [3]. At the same time, numerous factors related to the causes of infertility, the duration of treatment and psychological stress can affect the functional state of various physiological systems that ensure the adaptation of the body and the development of complications of the gestational process [4].

Consequently, during induced pregnancy, changes in the course of already existing psychological disorders occur. At the same time, the identification of depression in this category of women is difficult, since many symptoms, such as the lability of the emotional background, increased fatigue, changes in appetite and a decrease in cognitive functions, are also often encountered during physiologically normal pregnancy [5, 12].

The aim of study was to evaluate and determinate of special features of the psychological health of women with induced pregnancy and its relationship with nausea and vomiting.

## MATERIAL AND METHODS

### Ethical approval

The review board and ethics committee of Tashkent Institute for Post-Graduate Medical Education approved the study protocol and informed consents were taken from all the participants.

The study included 270 women with nausea and vomiting in the first trimester of induced pregnancy (NVIP). The psychological evaluation of pregnant women was carried out with the State-Trait Anxiety Inventory (STAI) developed by Charles D. Spielberger. According to Brunton et al. [1] in 41 studies, the Spielberger scale showed an average of 90% reliability in diagnosing the degree of anxiety in pregnant women.

The State-Trait Anxiety Inventory (STAI) measures two types of anxiety: state anxiety or anxiety about an event, and trait anxiety or anxiety level as a trait characteristic. Higher scores are positively correlated with higher levels of anxiety. The STAI is a test/questionnaire given to adults that shows how strong a person's feelings of anxiety are. Feelings of unease, worry, tension, and stress can be defined as anxiety. The STAI tests two different types of anxiety, state and trait anxiety.

Scores range from 20 to 80, with higher scores correlating with greater anxiety. The creators of this test separated the different anxieties so both scales would be reliable. This means the S-anxiety scale would only measure S-anxiety and the T-anxiety scale would only measure T-anxiety, the ultimate goal in creating this test. They found they could not achieve this if the questions were the same to examine both types of anxiety. Each scale asks twenty questions each and is rated on a 4-point scale [7, 13].

Low scores indicate a mild form of anxiety whereas median scores indicate a moderate form of anxiety and high scores indicate a severe form of anxiety. Both scales have anxiety absent and anxiety present questions. Anxiety absent questions represent the absence of anxiety in a statement like, "I feel secure." Anxiety present questions represent the presence of anxiety in a statement like "I feel worried."

More examples from the STAI on anxiety absent and present questions are listed below. Each measure has a different rating scale. The 4-point scale for S-anxiety is as follows: 1) not at all, 2) somewhat, 3) moderately so, 4) very much so. The 4-point scale for T-anxiety is as follows: 1) almost never, 2) sometimes, 3) often, 4) almost always.

## RESULTS

As can be seen from figure 1 during the psychological evaluation of anxiety, the distribution of patients according to the Spielberger scale was as follows:

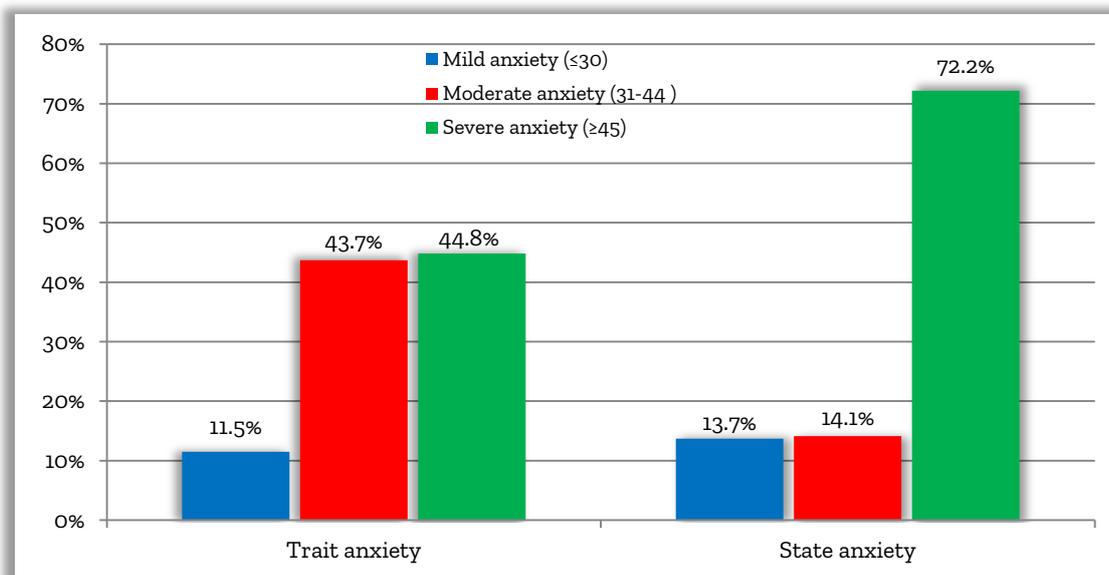
- Low anxiety occurred in 31 (11,5%) pregnant women with NVIP,
- In 118 (43,7%) cases a moderate level of trait anxiety was established,
- 121 (44,8%) women have a high level of anxiety.

According to our data, most of the surveyed women with NVIP experienced a high level of situational anxiety, which amounted to 72.2% (n = 195). For moderate and low degrees of state anxiety, approximately equal frequency was registered, 14.1% (n = 38) and 13.7% (n = 37), respectively.

Such a high rate of state anxiety in women with induced pregnancy is due to the fact that the symptoms of nausea and vomiting in the first trimester led to an increase in the initially existing anxiety and depression. Thus, state or situational anxiety with NVIP arose as an emotional reaction to nausea and vomiting, as to a stressful situation. At the same time, state anxiety, different in intensity and dynamism in time, was observed characteristic of induced pregnancy. In order to obtain the characteristics of the mental health of women with induced pregnancy and developed NVIP, we found it interesting to have a more detailed study of state anxiety.

In the course of studying the results of situational (state) psychodiagnostics on the Spielberger scale, the response rate "very much so" to the specific for anxiety syndrome of pregnant women with NVIP judgments was analyzed (Table 1).

The state of tension and regretful from the current situation was noted by 175 (64.8%), anxiety and nervousness were registered in 158 (58.5%) and 207 (76.7%) cases, respectively. 147 (54.4%) and 187 (69.3%) women, respectively, noted their excitement for possible failures and concern.



**Figure 1.** Distribution of STAI according to trait and state anxiety degree of women with NVIP

**Table 1.** The results of the situational (state) anxiety evaluation in pregnant women according to the Spielberger scale score STAI

Nº	Statements	Not at all	Somewhat	Moderately so	Very much so
1.	I feel calm	134	121	15	0
2.	I feel secure	135	129	6	0
3.	I am tense	0	39	56	175
4.	I am regretful	0	32	63	175
5.	I feel at ease	193	71	6	0
6.	I feel upset	0	77	78	115
7.	I am presently worrying about possible misfortunes	13	41	69	147
8.	I feel rested	197	37	36	0
9.	I feel anxious	4	43	65	158
10.	I feel comfortable	263	4	3	0
11.	I feel self-confident	195	39	36	0
12.	I feel nervous	0	27	36	207
13.	I am jittery	0	57	63	150
14.	I feel "high strung"	0	52	53	165
15.	I am relaxed	234	23	13	0
16.	I feel content	243	27	0	0
17.	I am worried	0	37	46	187
18.	I feel over-exited and rattled	0	54	79	137
19.	I feel joyful	237	33	0	0
20.	I feel pleasant	232	38	0	0

1) not at all, 2) somewhat, 3) moderately so, 4) very much so.

## DISCUSSION

Until recently, the assessment of the course of induced pregnancy was mainly reduced to the study of physiological processes and their corresponding complications, the biological side was mainly studied, and only in recent years did interest arise in the psychological problems of induced pregnancy. Also, in the majority of works on this topic, attention not paid to social aspects. It is believed that socially conditioned stress contributes to the development of psycho-emotional tension and the progression of symptoms of threatened abortion [9, 10].

As for the studies of the psycho-emotional status of pregnant women suffering from nausea and vomiting, they are not numerous, but researchers point to the existence of a close interdependence of mental functions and biological processes, having data on changes in biochemical parameters in the use of psychotherapy and improving the quality of life of patients [11].

State anxiety can be defined as fear, nervousness, discomfort, etc. and the arousal of the autonomic nervous system induced by different situations that are perceived as dangerous. This type of anxiety refers more to how a person is feeling at the time of a perceived threat and is considered temporary [6].

Trait anxiety can be defined as feelings of stress, worry, discomfort, etc. that one experiences on a day to day basis. This is usually perceived as how people feel across typical situations that everyone experiences on a daily basis.

State anxiety with nausea and vomiting induced pregnancy was manifested by tension, internal stiffness, anxiety, nervousness, and anxiety. During pregnancy, due to changes in hormonal and psychoemotional background in women with induced pregnancy, an increase in the level of state anxiety occurs. As the symptoms of the NVIP increase and worsen, the anxiety and stress increase.

## CONCLUSION

The study of the psycho-emotional state of women with NVIP revealed that 44.8% of them had a high level of trait anxiety, and 72.2% noted a rise in the level of situational anxiety, which arises as a result of the emotional reaction to NVIP. It should be noted that, regardless of the severity of NVIP during induced pregnancy, 87.7% of women report a feeling of tension and anxiety about abortion.

The data obtained from the high frequency of severe forms of NVIP and anxiety-depressive conditions among women with induced pregnancy serve as an argument for the early administration of psychologist's consultations, as one of the main links in the treatment of NVIP.

## DECLARATIONS

### Authors' contributions

All authors contributed equally to this work.

### Competing interests

The authors declare that they have no competing interests.

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# Impact of school meals' type and time on children's food consumption, physical and behavioral activities

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## ABSTRACT

Today, proper nutrition is one of the useful tools for the healthiness and sustainability of people's diet and especially student performance and behavior in school. Existing nutrient standards for school meals are always important when packed foods or lunches brought from home. The aim of the present study was to determine the effects of school meals type and time on behavioral response, physical activity and the body mass index (BMI) in elementary students of Razan city, Iran. Elementary school principals (N = 16) and total of 234 students selected from 5 schools completed a survey on the school food and physical and behavioral activities environment. Students were weighed and measured for their body mass index (BMI) that calculated using a standard protocol and also send a BMI report card to their parents. Results of this study showed that effect of type of time regulation in school nutritional program could significantly improve behavioral response, and especially physical activity and BMI and it can be considered as an applicable strategy to the implementation of such programs on the health status of students.

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Body mass index,  
Nutritional behavior,  
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## INTRODUCTION

To date, physical activity and nutritional intake are always one of the important factors of overweight and obesity, especially in students. Currently, wrong nutritional regimes, trends and the related disorders in advanced countries are as epidemiology [1, 2]. Recently studies have focused on the health status of students on behavioral and cognitive subjects linked to psychological - physical pathologies and mainly from view point of school nutritional program [2]. Results of early conducted study in Iran showed that almost eighteen percentages of teenagers were suffered from overweight and obesity [3] and from cardiovascular risks like high bad cholesterol or LDL-C (low density lipoprotein cholesterol) and those aged >6-12 years had high triglycerides and low good cholesterol (high DL-C) [4].

Cox et al. [5] referred to some problems as emotional failures, alcoholism, smoking, illegal drugs and eating disorders and reported that it seems that relationship between behaviors and nutrition are more complex than smoking and drinking alcohol, so that quantitative indices can used easily to assess Psychoactive drugs but nutritional behaviors are more complex and multidimensional in terms of evaluation [3]. Some indices as rate of the food intake and calorie, feelings of satiety, anorexia and overweight are various factors that evaluate some aspects of nutritional behaviors. One of the most acceptable and common indices in most of the researches is body mass index. This index is calculated via weight and height of the person and it is a rather reliable index of body fat in most of the people. It is worth to mention that BMI doesn't measure body fat directly, but the researches showed that BMI has linear correlation with direct evaluation of body fat such as weighting under water and dual energy x-ray absorptiometry [6]. BMI in most of the researches is related to a wide range of health issue (increasing risk of cardiovascular diseases, diabetic, cancer and arthritis) [7]. So BMI is considered as an important variable in health researches. Hence, one of the important scopes of experiments is the examination of the factors related to BMI.

In some of the studies, cognitive variables (memory, attention range etc.) are investigated as BMI predictive [8]. In other studies, some indices as food external index, emotional factors and abstain eating are supported as BMI predictive [9, 10]. Some of the researches also referred to some factors as stress, anxiety and

depression as probable predictor of BMI and moderator effects of some variables such as gender and physical activity [8]. In some of the researches, some variables such as knowledge, attitude and performance of guidance and high school students about healthy nutrition were investigated [11] so that they reported that a small percentage of teenagers have good nutritional performance and in most of the cases their attitude and performance is not according to their nutritional knowledge. Nutritional knowledge is not the only effective factor on nutritional behaviors [12, 13] and there are other variables that were more effective in this regard.

Subject of the current study was investigating effect of programming and intervention on nutritional time and type toward a better understanding behavior of students, their physical activity and BMI factors.

## MATERIAL AND METHODS

The presented study was conducted in Iranian elementary school principals (N=16) of Hamedan, Razan city, by participating 9, 10 and 11 years students (N=234) in a survey. A total number of twenty hundred thirty four male students (n=81, n=89 and n=64 for classes III, IV and V, respectively) were randomly selected from 5 schools. The students completed a simple survey on the school food and physical and behavioral activities environment in 2016–12.

Questionnaire and method of collecting data were used according to work of Azadbakht et al. [11]. Questionnaire was contained 21 items including, nutritional knowledge or behavior, attitude toward nutrition and their age and class. In order to study the body mass index of participants, their weight and height measured using a standard protocol. In order to answer the research question, the mean scores of respondent was presented as percentages of students as accepted or rejected program and its types and times.

### Ethical approval

The review board and ethics committee of Educational Administration of Razan, Iran approved the study protocol and informed consents were taken from all the participants.

### Statistical analysis

For analyze the data, in descriptive statistics section, the indexes of mean and standard deviation was calculated and in inferential section, and the one sample T test was used. All collected data were analyzed by V.16, SPSS statistical software (Predictive Analytics Software, PASW).

## RESULTS AND DISCUSSION

Descriptive statistics of the research variables were shown in Tables 1 and 2. To assess hypothesis of the current study, correlation between predictive matrices and criterion variables such as BMI was calculated. Percentage of Normal BMI for all students was 76.44 and as a consequence, nutritional behavior and attitude had the highest correlation with BMI variable while age variable showed the lowest correlation with BMI (Table 2).

School nutrition program was significantly ( $P<0.01$ ) accepted by all participants. That is showed a tendency and appetite in elementary students; so that it was desirable for students if nutrition implemented between lesson classes and in recess time or performed within second and third classes. Physical activity after school nutrition satisfied class V's students while those participated from classes III and IV were not acquiescent with this plan. Class III's students loved school nutrition twice in day while satisfactory decreased by class IV and V's students, significantly ( $P<0.01$ ). School nutrition in bell sports was not accepted by all of volunteers ( $P<0.01$ ).

The purpose of the presented study was investigating effect of programming and intervention on nutritional time and type in order to better understanding behavior and acceptability of program in elementary students, their physical activity and BMI factors.

According to the results of previous researches, some factors such as nutritional behavior, nutritional knowledge, attitude toward nutrition, physical exercise and demographic variables of age and gender were investigated as predictive variables of body mass index. In the current results, examined factors predicted normal BMI significantly which these results were supported hypotheses of the research.

Study of the results obtained from Sadrzadeh Yeganeh et al. [14] showed relationship of the obesity with some the nutritional behaviors among school girls. In another study [11], it was shown that despite having good nutritional knowledge, low values of nutritional behavior in high school students were detected. Likewise, results of the presented study revealed that predictive variable of nutritional behavior are referred as the most important BMI predictive variable. The various studies showed that nutritional behavior, nutritional knowledge, attitude toward nutrition and physical exercise are good variables to investigate the nutritional trend of elementary students and their weights [12, 15]. The results observed in the current study is similar to the findings of the same researches carried out in this field [16-18].

As presented in results section, a programmed nutritional time and type has good influence on promotion of behavior of elementary students and their physical activity and therefore culture of using the modern

educational tools and providing proper opportunities for enriching the student's scientific threshold via a proper and managed nutrition.

**Table 1.** Descriptive statistics of correlated variables of school meals

Nutritional Variables	M	SD	Min	Max
Age	10.5	2.15	9	11
Knowledge	8.24	1.05	5.5	9
Attitude	9.15	1.16	6	11
Behavior	6.5	1.60	4	8

**Table 2.** Descriptive statistics of categorical variables

Variables	Body Mass Index	Third Grade	Fourth Grade	Fifth Grade	Total	Normal	Abnormal
Frequency		81	89	64	234	172	55
Percentage		97	95	98	100	76.44	24.45

**Table 3.** Percentage and standard deviation of studied items and comparison between scores by single group T statistical test for students in three classes

Range of effectiveness	N	Percentage	SD	MD	T value	DF	Significance
<b>Third Grade</b>							
Acceptability of school meal program	81	91.3	4.58	2.68	6.64	80	P < 0.01
Meals between lesson classes and in recess time	81	93.82	5.53	4.95	9.76	80	P < 0.01
Meals within first and second classes	81	71.43	1.56	4.23	4.25	80	P < 0.08
Meals within second and third classes	81	60.49	2.46	3.56	5.51	80	P < 0.01
I love physical activity after school meal	81	37.03	1.63	2.37	3.26	80	P < 0.05
I love school meals twice in day	81	67.90	2.52	2.84	8.77	80	P > 0.05
I love school meals be in bell sports	81	25.92	1.53	2.68	7.06	80	P > 0.05
<b>Fourth Grade</b>							
Acceptability of school meal program	89	94.38	5.50	4.68	6.64	88	P < 0.01
Meals between lesson classes and in recess time	89	80.89	4.53	3.95	9.76	88	P < 0.01
Meals within second and third classes	89	73.03	4.56	3.56	5.51	88	P < 0.01
I love physical activity after school meal	89	37.03	3.63	2.37	3.26	88	P < 0.01
I love school meals twice in day	89	29.21	2.52	2.84	8.77	88	P < 0.01
I love school meals be in bell sports	89	42.69	3.53	3.68	7.06	88	P < 0.01
<b>Fifth Grade</b>							
Acceptability of school meal program	64	95.31	5.56	4.68	6.64	63	P < 0.01
Meals between lesson classes and in recess time	64	60.93	4.53	3.95	9.76	63	P < 0.01
Meals within second and third classes	64	54.68	3.56	2.56	5.51	63	P < 0.01
I love physical activity after school meal	64	78.12	4.63	2.37	3.26	63	P < 0.01
I love school meals twice in day	64	23.43	2.52	2.84	8.77	63	P = 0.10
I love school meals be in bell sports	64	10.93	1.25	1.68	7.06	63	P < 0.01
<b>Principals</b>							
Acceptability of school meal program	16	95.31	5.56	4.68	6.64	15	P < 0.01
Meals between lesson classes and in recess time	16	60.93	4.53	3.95	9.76	15	P < 0.01
Meals within second and third classes	16	54.68	3.56	2.56	5.51	15	P < 0.01
I love physical activity after school meal	16	78.12	4.63	2.37	3.26	15	P < 0.01
I love school meals twice in day	16	23.43	2.52	2.84	8.77	15	P < 0.01
I love school meals be in bell sports	16	10.93	1.25	1.68	7.06	15	P < 0.01

MD= Mean difference, DF= Degree of freedom, SD= Standard error

## CONCLUSION

Results showed that non-profit elementary schools had high influence and effectiveness on compensating the opportunities of education, reinforcement of scientific capacity and stamina of students, facilitating the scientific-investigative relations, providing new educational opportunities for employees, generalizing and

stabilizing a scientific-healthy educational system. Previous findings of studies made in this area are also in conformity with the results of the present survey.

In future researches, obstacles of using modern education-nutritional tools and also cognitive and emotional consequences of using remote education system can be examined. As a consequence, implementation of the self-report questionnaire, which was limited to managers and educational employees of elementary schools, should be under consideration.

## DECLARATIONS

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### Authors' contributions

All authors contributed equally to this work.

### Competing interest

The authors declare that they have no competing interests.

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# Analysis of heavy metal content of Cu, Pb, Hg and dissolved Sn in coastal of Banyuwangi district, Indonesia

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## ABSTRACT

Banyuwangi Regency has the longest coast in East Java of Indonesia with sandy beaches and corals and there are various types of coastal and marine resources that can be utilized both in terms of economics and environment. But in the current era of industrialization, coastal areas in Banyuwangi have become a top priority for industrial development, agribusiness, agro-industry, housing, transportation, ports and tourism. The purpose of this study was to analyze the content of copper (Cu), lead (Pb), mercury (Hg), and tin (Sn) and the effect of water quality on the heavy metal content in the coast of Banyuwangi Regency. The method in this study uses descriptive. Data taken along the coast of Banyuwangi Regency include water quality (alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature and salinity), copper (Cu), lead (Pb), mercury (Hg), and tin (Sn). Data analysis using multiple linear regression analysis, followed by F test and t-test. The results showed that there was an influence between the quality of the water on the value of heavy metal of copper (Cu), and the value of R-Square 0.681 which means that it has an influence proportion on the value of copper (Cu) of 68.1%. Likewise, for the quality of water for tin (Sn), there is an influence with the value of R-Square of 0.700, which means that the effect is as high as 70%. While the quality of the waters against Lead, heavy metal (Pb) and mercury (Hg) has no significant effect. Based on the results of the study, Banyuwangi district government needs to take serious actions in controlling heavy metal pollution through the implementation of law No. 23 of 1997 concerning to environmental management, and the application of environmental quality standards more strictly.

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Water quality

## INTRODUCTION

Like other coastal waters, the Coastal of Banyuwangi District has the potential to accumulate anthropogenic loads carried from several rivers. This is compounded by the misuse of the river as a waste disposal site so that the pollutant load will be distributed to the river estuary also to the sea. The input of waste from land to estuary generally comes from human activities such as industry, shipping, anthropogenic and others [1]. This makes estuary and coastal areas vulnerable to contamination [2].

Like fresh water, sea water also has a great ability to dissolve various substances, both in the form of gases, liquids, and solids. A sea is a place where the rivers transport various types of substances, which can be beneficial nutrients for fish and aquatic organisms, can also be materials that are not useful, even disrupt the growth and development of fish and aquatic organisms or can cause a decrease in water quality [3].

This decrease in water quality is caused by the presence of contaminants, both in the form of organic and inorganic components. Inorganic components include dangerous heavy metals. Darmono [4], explained that the definition of heavy metals is a metal element with a high molecular weight, which is specific gravity greater than 5 g/cm<sup>3</sup>. However metalloid elements which have dangerous properties are also included in the group. Thus, currently elements included in heavy metals reach approximately 40 types of elements.

One of the pollutants that has the potential to be found in the coastal district of Banyuwangi is heavy metal. Pollution of heavy metals is categorized as pollution which causes harmful effects on the environment and the organisms in it. Heavy metals have non-degradable properties. In addition, heavy metals will accumulate in the environment such as water and sediment columns and be absorbed into marine biota [5].

Heavy metals can enter the environment in various ways, such as weathering of rocks containing heavy metals, volcanic activity and disposal of waste from mining, industry and transportation. The main source of heavy metal contaminants comes from air and water that pollute the soil. Certain metals in high concentrations will be very dangerous if found in the environment. The main cause of heavy metals being dangerous pollutants is because they are non-degradable by living organisms in the environment. As a result, these metals

accumulate into the environment. Heavy metals are dangerous if they enter the metabolic system in amounts exceeding the threshold. The threshold varies for each type of heavy metal [4]. Some of them are widely used in various daily needs; therefore they are produced regularly on an industrial scale. The use of these heavy metals in various daily needs, either directly or indirectly, or intentionally or unintentionally, has polluted the environment. Some heavy metals that are dangerous and often pollute the environment are mainly mercury (Hg), lead (Pb), arsenic (As), copper (Cu), cadmium (Cd), chromium (Cr), and nickel (Ni) [6].

Cu is a microelement is needed by organisms of both land and water, but in small amounts. The presence of Cu in general waters can come from industrial areas around the waters. This metal will be absorbed by aquatic biota sustainably if its presence in the water is always available, moreover, for aquatic biota with low mobility such as shellfish [7]. Lead (Pb) is gray metal, can be forged and can be formed. Pb has active chemical properties so that it can be used to coat metal to prevent corrosion. When mixed with other metals, lead can form better mixed metals than pure metal. In addition, lead also has a density exceeding other metals. This metal is widely used in the battery, cable, paint (as a coloring agent), gilding, pesticide industry and is the most widely used as an anti-dust agent in gasoline. Lead is also used as a constituent substance and as a pipe connecting formulation [4]. Tin (Sn) is a silvery white, shiny metal, can be forged and can be formed. Tin melting point is 231,930C. This metal is not easily oxidized in the air so it is often used as another metal coating to prevent rust. Tin is also often used as another metal coating to prevent rust. Tin is also often used as a mixture with other metals such as soft solder [8].

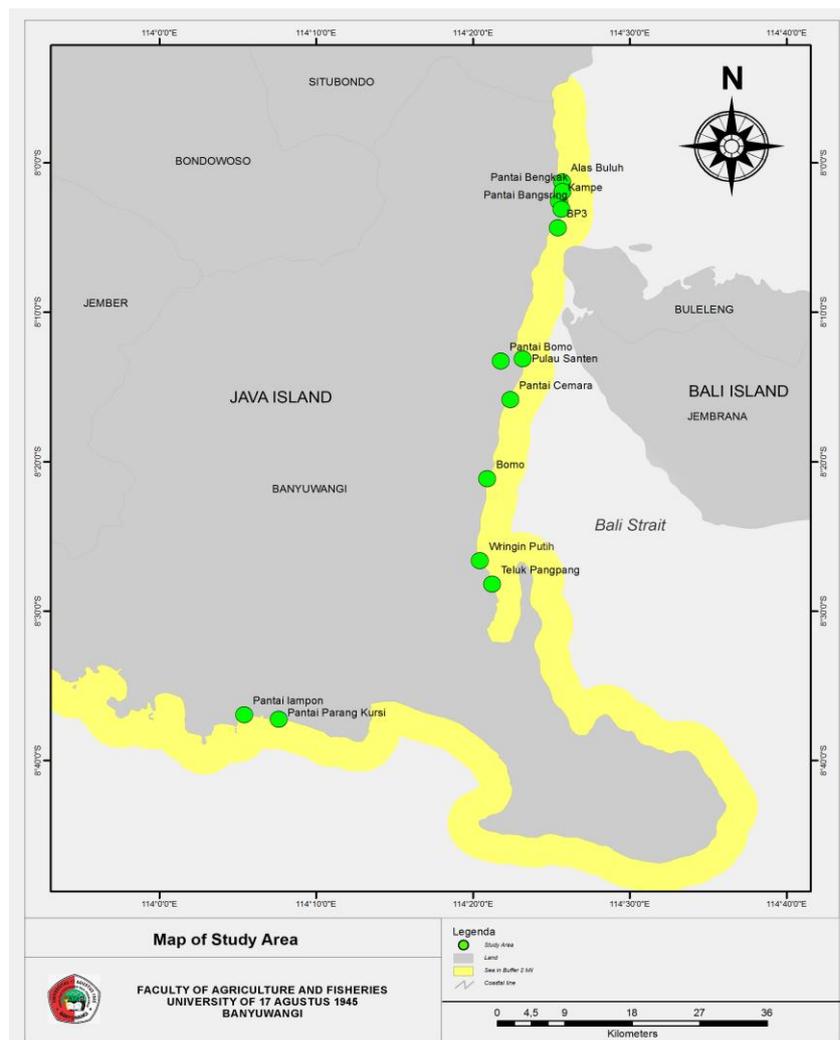
The presence of heavy metal at high concentration in the water column will endanger marine aquatic organisms from inhibiting metabolic process to causing the death of biota [9]. Therefore, this study aims to monitor the concentration of dissolved heavy metal along coastal of Banyuwangi district and analyze its association with aquatic environmental factors.

## MATERIAL AND METHODS

This research was conducted in March - June 2018. Data collection methods used purposive sampling along the coast of Banyuwangi Regency. The location of the study can be seen in Figure 1.

The research method uses descriptive methods, which is data presented by explaining and describing the real situation. Measurement of water quality which includes temperature, salinity, pH, dissolved oxygen (DO) was measured directly at the temporary research location for observing alkalinity,  $\text{NH}_4$ ,  $\text{PO}_4$ ,  $\text{NO}_3$  carried out at the faculty of agriculture and fisheries laboratory on university 17 August 1945 Banyuwangi. While taking water samples for heavy metals using dark glass bottles at each research location point along the coast of Banyuwangi Regency, then taken to the Surabaya to measure heavy metal levels.

Work procedure for analysis of heavy metal copper (Cu) content test method using Standar Nasional Indonesia (SNI) 6989.6: 2009, Mercury (Hg) using SNI 6989.78: 2011 test, Lead (Pb) using SNI 6989.46: 2009 test method, while Tin (Sn) using test method American Public Health Association (APHA) Ed. 21,311 B, 2005.



**Figure 1.** Banyuwangi Coastal Research Site Map.

Data analysis using multiple linear regression analysis to determine the degree of influence between variables of water quality and heavy metals. The statistical test results are presented in the form of mathematical equations, namely the multiple linear regression equation as follows:

$$Y = a + b_1X_1 + b_2X_2 + \dots + b_nX_n$$

Where:

Y: Dependent variable, a: Constanta, b<sub>1</sub>, b<sub>2</sub>: Regression coefficient, X<sub>1</sub>, X<sub>2</sub>: Independent variable

## RESULTS AND DISCUSSION

### Water quality parameters

Water quality data taken in the form of temperature, salinity, pH, DO and NH<sub>4</sub> in the waters of Banyuwangi coastal with the location of data collection in nine points representing all sub-districts along Banyuwangi coastal with twice replications.

Most of the water quality parameters can affect the concentration, distribution and toxicity of heavy metals in the waters referring to Hutagaol [10], which stated that temperature, turbidity, pH, salinity and DO are parameters that affect the toxicity of heavy metals in the waters. Environmental parameters are suspected affect heavy metal concentrations such as temperature, pH and salinity. The increase in temperature will reduce the adsorption of heavy metal compounds in particulates to settle to the bottom. The increase in pH can reduce the solubility of heavy metals in water because there is a change from the form of carbonate to hydroxide which forms a bond with particles in the water. Increasing salinity causes a decrease in toxic metals due to the desalination process. So, the existing heavy metal compounds can occur in the sedimentation process [11].

**Table 1.** Water quality data of Banyuwangi beach in 2018

Research Sites	Water Quality							Alkalinity	
	DO	Water Temperature	Water pH	Salinity	NH <sub>4</sub> (ppm)	NO <sub>3</sub> (ppm)	PO <sub>4</sub> (ppm)	CO <sub>3</sub> (ppm)	HCO <sub>3</sub> (ppm)
Alas Buluh	6.7	30.3	7.6	26	0	1	0	12	116
	6.5	29.7	7.3	25	0	0	0	36	112
Kampe	7.1	30.1	7.3	22	0	0	0	24	100
	7	28.8	7	23	0	0	0.1	12	144
BP3	8	27.3	7.2	20	0	0	0	24	116
	6.1	27.5	7.1	20	0	0	0.1	16	136
Cemara Beach	7.5	31	9	25	0	0	0.1	80	88
	6.4	29.3	8	25	0	0	0	32	100
Pakem Kertosari	7.7	31.6	7.2	24	0	0	0	12	112
	7.4	29.3	7.4	27	0	0	0	16	124
Santen Island	7.2	29.7	7.2	26	0	0	0	36	92
	6.5	29.2	7.4	26	0	0	0	36	100
Blimbingsari	6.1	30.3	8.9	27	0	0	0.1	12	120
	6.4	30.1	8.4	27	0	0	0.1	12	140
PangpangBay	7.04	30.3	6.9	23	0.7	0	0	24	98
	0.9	29.17	0	18	0.8	0	0	24	116
Lampon	6.9	30.6	7.1	25	0	0	3	44	84
	6.8	30.7	6.9	26	0	0	0	44	80

DO = dissolved oxygen; NH<sub>4</sub>= ammonium; PO<sub>4</sub>= phosphate; CO<sub>3</sub>= carbonate; HCO<sub>3</sub>= bicarbonate; BP3= Balai Pendidikan dan Pelatihan Perikanan (Fisheries Education and Training Center)

### Heavy metals Cu, Hg, Pb and Sn in the coastal of Banyuwangi regency

The heavy metals analyzed in this study were types of Copper (Cu), Mercury (Hg), Lead (Pb) and tin (Sn). The following are the results of heavy metal tests carried out in the Surabaya Industrial Research and Standardization Center laboratory.

In general, the range of Cu concentration is 0.0104 mg/l, Hg 0 mg/l, Pb 0.0173 mg/l and Sn 1.3436 mg/l obtained from the coastal waters of Banyuwangi Regency. If referring to the Decree of the Minister of Environment No. 51 of 2004 concerning Sea Water Quality Standards, Mercury (Hg) 0.001 mg/l, Copper (Cu) and Lead (Pb) 0.008 mg/l, and Tin (Sn) 2 mg/l, then the value of heavy metals Hg and Sn is still below the threshold while Cu and Pb are above the threshold.

According to WHO, the highest desirable level in drinking water for Cu is 50 µg/L and the threshold for concentration for aquatic life tolerance (safe for most fishes) is  $2 \times 10^4$  µg/L [12]. As for Pb, the WHO maximum permissible level of drinking water is 100 µg/L and the threshold of concentration for aquatic life tolerance (safe for most fishes) is 100 µg/L [12].

**Table 2.** Test results for heavy metals Cu, Hg, Pb and Sn in Coastal of Banyuwangi regency in 2018.

Parameter	Unit	Test Result						Test Methods
		P. 2169 Alas buluh	P. 2170 Alas buluh	P. 2171 Kampe	P. 2172 Kampe	P. 2173 BP 3	P. 2174 BP 3	
Copper (Cu)	mg/l	<0.0223	0.026	0.032	<0.0223	0.026	0.026	SNI 6989.6 : 2009
Mercury (Hg)	mg/l	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	SNI 6989.78 : 2011
Lead (Pb)	mg/l	0.012	0.015	0.015	0.016	0.017	0.015	SNI 6989.46:2009
Tin (Sn)*	mg/l	<0.1050	<0.1050	0.469	<0.1050	<0.1050	<0.1050	APHA Ed.21.311 B.2005

Parameter	Unit	Test Results						Test Methods
		P. 2175 P. Santen	P. 2176 P. Santen	P. 2177 P. Pakem	P. 2178 P. Pakem	P. 2179 P. Cemara	P. 2180 P. Cemara	
Copper (Cu)	mg/l	<0.0223	<0.0223	0.026	<0.0223	0.03	<0.0223	SNI 6989.6 : 2009
Mercury (Hg)	mg/l	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	SNI 6989.78 : 2011
Lead (Pb)	mg/l	0.017	0.018	0.017	0.022	0.018	0.018	SNI 6989.46:2009
Tin (Sn)*	mg/l	<0.1050	<0.1050	<0.1050	<0.1050	<0.1050	4.136	APHA Ed.21.311 B,2005

Parameter	Unit	Test Results				Test Methods
		P. 2181 Teluk Pampang	P. 2182 Lampon	P. 2183 Lampon	P. 2184 P. Blimbing Sari	
Copper (Cu)	mg/l	<0.0223	<0.0223	<0.0223	<0.0223	SNI 6989.6 : 2009
Mercury (Hg)	mg/l	<0.0005	<0.0005	<0.0005	<0.0005	SNI 6989.78 : 2011
Lead (Pb)	mg/l	0.018	0.019	0.021	0.018	SNI 6989.46:2009
Tin (Sn)*	mg/l	4.703	4.791	3.743	3.656	APHA Ed.21.311 B,2005

Note: Parameters tested according to parameters; "<" shows the limit of quantity value from the test

Source of heavy metals on the coast can be divided into two, which enter naturally and artificially into marine waters. Heavy metals that enter the ocean waters can come from three sources, namely:

- Input from the coastal area, which originates from the river and the results of coastal abrasion due to wave activity.
- Inputs from the deep sea, including metals released as a result of volcanic activity in deep seas and metals released from particles through chemical processes.
- Inputs from nearshore land environments, including metals originating from the atmosphere as dust particles.

The source of artificial metals is metal that was released during the metal and rock industry process. Some industries only use certain heavy metals for their production activities. However, in general, most industries use various types of heavy metal elements, making it difficult to trace the origin of sources of pollution. Of the four heavy metals mentioned above, different concentrations of heavy metals are obtained in seawater. This difference in concentration is possible due to the variability of metals in water caused by currents, adsorption, tides, or deposition [13].

#### Effect of water quality to heavy metal

Based on the results of regression analysis of the four types of heavy metals namely Copper (Cu), Lead (Pb), Mercury (Hg) and Lead (Pb) on the coast of Banyuwangi Regency, it shows that there is no effect on water quality of Lead (Pb) and Mercury (Hg). Whereas two other types of heavy metals, namely Copper (Cu) and Tin (Sn) have influence.

In connection with this, even though the Pb value is at the threshold, it is not caused by the value of water quality, but the presence of waste entering the coastal area. Whereas the Hg type value is below the threshold and the waste entering the coastal area means that it still contains Hg. According to Anggoro [14], heavy metal is one of the waste parameters as a source of impact in coastal waters. Probability value of calculated F (sig.) in the table above, the value 0,0001 is smaller than the significance level of 0.05 so it can be concluded that the linear regression model that was estimated is worth using to explain the effect of heavy metal copper (Cu) on alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, and salinity. Copper (Cu) is one of the heavy metals that can be found in the aquatic environment and in sediments [15]. Heavy metals naturally have low concentrations in waters. High or low concentrations of heavy metals are caused by the maximum amount of heavy metal waste into the waters. Heavy metals that enter the waters will experience precipitation, dilution and dispersion, then absorbed by organisms that live in the waters. Maslukah [16], states that the process of entering Cu in subsequent waters undergoes an adsorption process followed by a process of flocculation and desorption. The adsorption process by particles causes the precipitation of material in the sediment and makes the concentration near the bottom of the water column become high again.

From the Table 4, the R-Square value of 0.681, it shows that the proportion of the copper (Cu) variable influence to the variables of alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, and salinity is 68.1%. This means, that the value of the independent variable has an influence proportion on the value of copper (Cu) of 68.1% while the remaining 31.9% (100% - 68.1%) is influenced by other variables that are not in the linear regression model.

**Table 3.** F-Test: Water quality to copper (Cu) heavy metal

ANOVA <sup>a</sup>						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	0.0001	8	0.0001	8.265	0.000b
	Residual	0.0001	31	0.0001		
	Total	0.0001	39			

a. Dependent Variable: Tin (Cu); b. Predictors: (Constant), Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, Salinity

**Table 4.** R Square value: copper (Cu) heavy metal to water quality

Model Summary <sup>b</sup>					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.825a	0.681	0.598	0.0017955	2.587

a. Predictors: (Constant), Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, Salinity; b. Dependent Variable: Copper (Cu)

**Table 5.** t-Test: Heavy metal copper (Cu) towards water quality

Coefficients <sup>a</sup>								
Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity statistics		
	B	Std. Error	Beta			Tolerance	VIF	
1	(Constant)	-0.014	0.011		-1.289	0.207		
	DO	0.000	0.001	0.079	0.683	0.500	0.762	1.312
	water temperature	0.002	0.000	0.620	4.258	0.000	0.485	2.060
	pH	0.001	0.001	0.182	1.496	0.145	0.692	1.445
	Salinity	-0.001	0.000	-0.928	-5.907	0.000	0.417	2.396
	NH <sub>4</sub>	-0.011	0.002	-0.688	-5.193	0.000	0.586	1.707
	NO <sub>3</sub>	-0.004	0.001	-0.445	-3.387	0.002	0.597	1.674
	PO <sub>4</sub>	-0.001	0.000	-0.202	-1.882	0.069	0.895	1.118
	Alkalinity	4.021E-005	0.000	0.231	1.711	0.097	0.567	1.764

a. Dependent variable: copper (Cu); DO= dissolved oxygen; NH<sub>4</sub>= ammonium; NO<sub>3</sub> = nitrate ; PO<sub>4</sub>= phosphate; VIF: Variance Inflation F=actor

The probability value of calculated t from the independent variables of dissolved oxygen (DO) is 0.50, pH of 1.45, PO<sub>4</sub> and alkalinity of 0.097 (greater than Sig. 0.05) indicates that the independent variable dissolved oxygen (DO) , pH, PO<sub>4</sub>, and alkalinity have no significant effect on the dependent variable of copper (Cu). The probability value of calculated t from the independent variable water temperature of 0.00, salinity of 0.00, NH<sub>4</sub> of 0.00 and NO<sub>3</sub> of 0.02 (smaller than Sig. 0.05), indicating that the variable is independent of water temperature, salinity , NH<sub>4</sub>, and NO<sub>3</sub> have a significant effect on the dependent variable of copper (Cu).

Based on the above values, the interpretation of the models of alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, and salinity of Copper (Cu) heavy metals is as follows:

$$\text{Copper (Cu)} = -0.14 + 0.00 \text{ DO} + 0.002 \text{ Water Temperature} + 0.001 \text{ pH} - 0.001 \text{ salinity} - 0.011 \text{ NH}_4 - 0.004 \text{ NO}_3 - 0.001 \text{ PO}_4 + 4.021 \text{ alkalinity}$$

The regression coefficient of dissolved oxygen (DO) is positive, meaning that when the value of DO rises, the value of heavy metals Copper (Cu) will also increase. If the value of DO decreases, the value of Cu will decrease too. If the value of dissolved oxygen (DO) rises by 1 mg/l it will increase the total Cu value by 0,000 mg/l and conversely the decrease in DO by 1 mg/l will reduce the copper (Cu) value by 0,000 mg/l.

Water temperature regression coefficient is positive, meaning that when the water temperature rises, the copper (Cu) value will also increase. If the value of the water temperature drops, the value of copper (Cu) will decrease. If the value of the water temperature rises by 1 °C, it will increase the value of Cu by 0.002 mg/l and conversely a decrease in water temperature of 1 °C will reduce the value of Cu by 0.002 mg/l.

PH regression coefficient is positive, meaning that when the pH value rises, the value of copper (Cu) will also increase. If the pH value decreases, the copper (Cu) value will decrease. If the pH value rises by 1, it will increase the Cu value by 0.001 mg/l and conversely a decrease in pH of 1 will decrease the value of Cu by 0.001 mg/l.

Alkalinity regression coefficient has a positive value, meaning that when the alkalinity value rises, the copper (Cu) value will also increase. If the value of alkalinity decreases, the value of copper (Cu) will decrease. If the alkalinity value rises by 1, it will increase the Cu value by 4.021 mg/l and conversely a decrease in alkalinity of 1 will reduce the Cu value by 4.021 mg/l.

The salinity regression coefficient is negative, meaning that when the salinity value increases, the copper (Cu) value will decrease, whereas when the salinity value drops, the value of copper (Cu) will increase. If the salinity value increases by 1 then it will reduce the copper (Cu) value by 0.001 mg/l and conversely a decrease in the salinity value of 1 will increase the value of Cu by 0.001 mg/l.

This is confirmed also by [Balasubramanian \[17\]](#), that dissolve Cd and Pb in the coastal water indicated that salinity played a major role in the depletion of the dissolved metals during estuarine mixing. As salinity increased, the concentrations of dissolved Pb and Cd decreased. The data revealed that large quantum of metals was removed from the water column and precipitated as a suspended matter which may contaminate the bottom sediments. The decrease in the concentration of heavy metals with salinity showed the contribution from freshwater sources was insignificant which indicated that point sources and physical mixing of anthropogenic inputs injected by industrial, harbour activity, sewage etc. regulated the metal concentrations along these waters.

NH<sub>4</sub> regression coefficient is negative, meaning that when the value of NH<sub>4</sub> increases, the value of copper (Cu) will decrease, whereas when the value of NH<sub>4</sub> drops, the value of Cu will increase. If the value of NH<sub>4</sub> increases by 1 mg/l it will reduce the value of copper (Cu) by 0.011 mg/l and conversely the decrease in the value of NH<sub>4</sub> by 1 mg/l will increase the value of Cu by 0.011 mg/l.

NO<sub>3</sub> regression coefficient is negative, meaning that when the NO<sub>3</sub> value increases, the Cu value will decrease, whereas when the NO<sub>3</sub> value drops, the Cu value will increase. If the NO<sub>3</sub> value increases by 1 mg/l it will reduce the value of Cu by 0.004 mg/l and conversely a decrease in NO<sub>3</sub> value of 1 mg/l will increase the value of Cu by 0.004 mg/l.

PO<sub>4</sub> regression coefficient is negative, meaning that when the PO<sub>4</sub> value rises, the value of Cu will decrease, whereas when the PO<sub>4</sub> value drops, the value of Cu will increase. If the PO<sub>4</sub> value increases by 1 mg/l it will reduce the value of Cu by 0.001 mg/l and conversely the decrease in the value of PO<sub>4</sub> by 1 mg/l will increase the value of Cu by 0.001 mg/l.

According to [Balasubramanian \[17\]](#), Metals such as Zn, Mn, Cu, Cd, Hg, Pb, silt, clay, organic carbon (OC), pH and salinity with a strong factor loading (> 0.700) found to be a significant parameters contributing to the water quality of these coastal waters. High and positive scores of dissolved metals and sediment characteristic on variable 1 or 2 indicated high anthropogenic inputs from catchments. The presence of multiple variables present in the same factor suggested a close association among them and identical source.

The probability value of F count (Sig.) in the table above, the value is 0.270 greater than the significance level of 0.05 so it can be concluded that alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, salinity have no effect on lead (Pb) in coastal of Banyuwangi Regency.

The probability value of F count (Sig.) in the table above is 0.221 greater than the significance level of 0.05 so it can be concluded that alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, and salinity have no effect on mercury value (Hg).

The concentration of mercury (Hg) at each sampling location when the research was carried out was the same i.e. <0.0005 mg/l. This value based on the Decree of the State Minister of Environment Number 51 of 2004 concerning Sea Water Quality Standards is classified as very low and does not interfere with aquatic biota, including fish; which has been determined the threshold value is 0.001 mg/l. [Komarawidjaja \[19\]](#), explained that the value of the measurement results is still far below the quality standards that apply to any designation so that coastal waters are safe to be used as ponds, ports or marine tourism.

The probability value of counted F (Sig.) in the table above is 0.00 less than the significance level of 0.05, so that it can be concluded that the multiple linear regression model that is estimated is feasible to use to explain the effect of tin (Sn) on Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, and salinity.

From Table 8, the value of R-Square which is 0.700, it shows that the proportion of the influence of the variable tin (Sn) on the variables Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, and salinity by 70%. That is, the value of Sn has a proportional effect on Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, and salinity by 70% while the remaining 30% (100% - 70%) is influenced by other variables that do not exist in a linear regression model.

**Table 6.** F-Test: Water quality for lead metal (Pb)

ANOVA <sup>a</sup>						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	0.0001	8	0.0001	1.322	.270 <sup>b</sup>
	Residual	0.0001	31	0.0001		
	Total	0.0001	39			

a. Dependent Variable: Lead (Pb); b. Predictors: (Constant), Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, salinity

**Table 7.** F-Test: Water quality against mercury (Hg)

ANOVA <sup>a</sup>						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	0.0001	8	0.0001	1.437	0.221 <sup>b</sup>
	Residual	0.0001	31	0.0001		
	Total	0.0001	39			

a. Dependent Variable: Mercury (Hg); b. Predictors: (Constant), Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, salinity

**Table 8.** F-Test: Heavy metal tin (Sn) towards water quality

ANOVA <sup>a</sup>						
Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	113.151	8	14.144	9.049	0.0001 <sup>b</sup>
	Residual	48.452	31	1.563		
	Total	161.603	39			

a. Dependent Variable: Tin (Sn); b. Predictors: (Constant), Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, water temperature, salinity

**Table 9.** R Square value: heavy metal of tin (Sn) against water quality

Model Summary <sup>b</sup>					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	0.837a	0.700	0.623	1.2501901	1.453

a. Predictors: (Constant), Alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, Salinity; b. Dependent Variable: Tin (Sn)

**Table 10.** t Test: Heavy metal of tin (Sn) against water quality

Coefficients <sup>a</sup>							
Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity statistics	
	B	Std. Error	Beta			Tolerance	VIF
(Constant)	7.143	7.425		0.962	0.343		
DO	-1.147	0.454	-0.285	-2.527	0.017	0.762	1.312
water temperature	0.264	0.264	0.141	0.998	0.326	0.485	2.060
pH	0.527	0.388	0.160	1.356	0.185	0.692	1.445
1 Salinity	-0.056	0.115	-0.074	-0.483	0.632	0.417	2.396
NH <sub>4</sub>	4.329	1.520	0.366	2.848	0.008	0.586	1.707
NO <sub>3</sub>	-1.352	0.773	-0.222	-1.748	0.090	0.597	1.674
PO <sub>4</sub>	0.904	0.322	0.292	2.806	0.009	0.895	1.118
Alkalinity	-0.063	0.016	-0.499	-3.822	0.001	0.567	1.764

a. Dependent variable: tin (Sn); DO= dissolved oxygen; NH<sub>4</sub>= ammonium; NO<sub>3</sub> = nitrate ; PO<sub>4</sub>= phosphate; VIF: Variance Inflation F=actor

The probability value of counted t from the independent variable of water temperature is 0.326, pH is 0.185, salinity is 0.632, and NO<sub>3</sub> is 0.09 (greater than Sig. 0.05) indicating that the independent variables of water temperature, pH, salinity and NO<sub>3</sub> are not significant effect on the dependent variable tin (Sn). The probability value of t count variable dissolved oxygen (DO) is 0.017, NH<sub>4</sub> is 0.08, PO<sub>4</sub> is 0.009, and alkalinity is 0.01 (p < 0.05), indicating that the independent variable DO, NH<sub>4</sub>, PO<sub>4</sub>, and alkalinity have a significant effect on the dependent variable tin (Sn). Based on the above values, the interpretation of the models of alkalinity, NH<sub>4</sub>, PO<sub>4</sub>, DO, pH, NO<sub>3</sub>, Water Temperature, and Salinity for tin heavy metal (Sn) are as follows:

$$\text{Tin (Sn)} = 7,143 - 1,147 \text{ DO} + 0,264 \text{ Water Temperature} + 0,527 \text{ pH} - 0,56 \text{ Salinity} + 4,329 \text{ NH}_4 - 1,352 \text{ NO}_3 + 0,904 \text{ PO}_4 - 0,063 \text{ alkalinity.}$$

Water temperature regression coefficient is positive, meaning that when the water temperature rises, the value of tin (Sn) will also increase. If the value of the water temperature drops, the value of Sn will decrease. If the value of the water temperature rises by 1 °C, it will increase the value of tin (Sn) by 0.264 mg/l and conversely a decrease in water temperature of 1 °C will reduce the value of Sn by 0.264 mg/l. The pH regression coefficient is positive, meaning that when the pH value rises, the value of tin (Sn) will also increase. If the pH value drops, the value of tin (Sn) will decrease. If the pH value increases by 1, it will increase the value of Sn by 0.527 mg/l and conversely a decrease in pH of 1 will decrease the value of Sn by 0.527 mg/l.

NH<sub>4</sub> regression coefficient is positive, meaning that when the value of NH<sub>4</sub> rises, the value of tin (Sn) will also increase. If the value of NH<sub>4</sub> drops, the value of tin (Sn) will decrease. If the value of NH<sub>4</sub> rises by 1 mg/l, it will increase the value of Sn by 4.329 mg/l and conversely the decrease in NH<sub>4</sub> by 1 mg/l will reduce the value of tin (Sn) by 4.329 mg/l.

PO<sub>4</sub> regression coefficients are positive, meaning that when the PO<sub>4</sub> value rises, the value of tin (Sn) will also increase. If the PO<sub>4</sub> value drops, the value of Sn will decrease. If the value of PO<sub>4</sub> rises by 1 mg/l, it will increase the value of Sn by 0.904 mg/l and conversely a decrease in PO<sub>4</sub> of 1 mg/l will decrease the value of Sn by 0.904 mg/l. The regression coefficient of dissolved oxygen (DO) is negative, meaning that when the dissolved oxygen value rises, the value of Sn will decrease, whereas when the DO value drops, the value of Sn will increase. If the value of DO increases by 1 mg/l it will reduce the value of Sn by 1.147 mg/l and conversely a decrease in DO of 1 mg/l will increase the tin value by 1,147 mg/l. The salinity regression coefficient is negative, meaning that when the salinity value rises, the value of tin (Sn) will decrease, whereas when the salinity value drops, the value of tin (Sn) will increase. If the salinity value increases by 1 ppt it will reduce the value of Sn by 0.056 mg/l and conversely a decrease in the salinity value of 1 ppt will increase the value of Sn by 0.056 mg/l. NO<sub>3</sub> regression coefficient is negative, meaning that when the NO<sub>3</sub> value rises, the value of Sn will decrease, whereas when the NO<sub>3</sub> value drops, the value of Sn will increase. If the NO<sub>3</sub> value increases by 1 mg/l it will reduce the value of Sn by 1.352 mg/l and conversely a decrease in NO<sub>3</sub> value of 1 mg/l will increase the value of Sn by 1.352 mg/l.

The regression coefficient of alkalinity is negative, meaning that when the alkalinity value rises, the value of Sn will decrease, whereas when the value of alkalinity decreases, the value of Sn will increase. If the alkalinity value increases by 1 mg/l it will reduce the value of Sn by 0.063 mg/l and vice versa the decrease in the value of alkalinity by 1 mg/l will increase the value of Sn by 0.063 mg/l. The high pH and low dissolved oxygen content in this sampling site can contribute towards this situation. Hot Spring waters of Lake Bogoria (BG1), Lake Elementaita (EL1) contained lower concentrations of heavy metals. High pH and temperature and very low oxygen can encourage solubilization processes and subsequent precipitation [18].

## CONCLUSION

The concentrations of Cu, Hg, Pb and Sn obtained in the coastal waters of Banyuwangi Regency were Cu 0.0104 mg/l, Hg 0 mg/l, Pb 0.0173 mg/l and Sn 1.3436 mg/l. If referring to the Keputusan Menteri Lingkungan Hidup (Decree of the Minister of Environment) No. 51 of 2004 concerning Sea Water Quality Standards, Mercury (Hg) 0.001 mg/l, Copper (Cu) and Lead (Pb) 0.008 mg/l, and Tin (Sn) 2 mg/l, then the value of heavy metals Hg and Sn is still below the threshold while Cu and Pb are above the threshold. Whereas based on the results of regression analysis, of the four types of heavy metals Copper (Cu), Lead (Pb), Mercury (Hg) and Lead (Pb) on the coast of Banyuwangi Regency, indicating water quality that there is no effect on Lead specific gravity metals (Pb) and Mercury (Hg); whereas two other types of heavy metals Cu and Sn had influence.

Based on the results of the study, Banyuwangi district government needs to take serious actions in controlling heavy metal pollution through the implementation of law No. 23 of 1997 concerning to environmental management, and the application of environmental quality standards more strictly.

## DECLARATIONS

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### Authors' contributions

All authors contributed equally to this work.

### Competing interests

The authors declare that they have no competing interests.

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# New hemostatic preparation made of the cellulose derivatives

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## ABSTRACT

**Aim.** The aim of this study was to investigate the indexes the indexes of biocompatible pellicle hemostatic coverage *in vitro* and *in vivo* conditions. **Methods.** Samples of pellicle hemostatic coverage on the basis of the cellulose derivatives were used in researches. Breaking strength, estimation of the implant's structure and adhesion power were evaluated according to the Ts 05957837-28:2014 instructions and documents of the National certification system of the Republic of Uzbekistan and with the using the apparatus "Zwick" (Germany) and atomic-power microscope of Agilent technologies (USA). Hemostatic activity of the coverage on the basis of the cellulose derivatives was estimated by the Lee and White test for the blood coagulation time. For *in vivo* research, 30 mature rats were required. Operations were performed under inhalation anesthesia, and the wound of liver was formed. Both macroscopic and microscopic studies had been undertaken. Morphological changes were studied in terms of 3 and 12 hours and then on the 1<sup>st</sup>, 3<sup>d</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 30<sup>th</sup> day after an operation. **Results.** An adhesion power of the pellicle coverage on the basis of the cellulose derivatives was  $7.3 \pm 0.2$  N/cm<sup>2</sup>, breaking strength was  $390 \pm 4.8$  kGf/cm<sup>2</sup>. In presence of polymer, a coagulation time on Lee and White test was shortened by as many as 2.1 times in relation to control that made up  $2.4 \pm 0.6$  min. In *in vivo* conditions hemostasis started during 3-5 sec. A weak inflammatory reaction of tissue was histologically determined. Further observations over dogs showed that an hour after an operation, an implant had been preserved on the surface of liver as a white pellicle and had not been separated from the wound surface. Bleeding signs were not marked. An abdominal cavity remained intact. **Conclusion.** Rapid enough biodegradation of polymer along with the unexpressed inflammatory reaction allows preventing the infecting related to the presence of foreign body. The rapid forming of fibrotic tissue in a zone of lesion makes it possible to obtain a durable hemostasis. A poorly expressed reaction was also marked from the side of peritoneum and surrounding organs. **Recommendations.** The oxidized regenerated cellulose can result in an intensive inflammation of the surrounding tissues because of the low level of pH that had not happened in the present research due to the selected correlation of ingredients of hemostatic pellicle.

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## INTRODUCTION

Cellulose is a natural polymer of vegetable origin, not water-soluble. It possesses a good biocompatibility, does not cause an allergic reaction, and in this connection is widely used in medicine and pharmaceutical industry. Oxidized regenerated cellulose (ORC) – is the water-soluble cellulose's derivative which is synthesized by the influence of oxidizing agents. ORC in the organism of a human biodegrades without the forming the toxic substances and does not cause the expressed inflammatory reaction of tissue [1]. Additionally ORC possesses an antibacterial effect initiating the forming the unfavorable pH environment. Bio-absorption of ORC proceeds during two weeks [2]. ORC is successfully used in plastic surgery because it does not cause an edema or deformation of tissue [3]. Mechanisms of the favorable healing and fibrogenesis are explained by the ORC's absorption of free radicals, ions of metals, stabilizing of growing factors and others [4].

Carboxymethylcellulose (CMC) is a nontoxic and biocompatible polymer owing to what it finds a wide use in pharmaceutical, cosmetic, and food industry [5-7]. Ca-CMC unlike Na-CMC is not dissolved to the end in water, swells and forms a gel substance [8]. The mechanism of hemostatic action of Ca-CMC is in a point that at

a contact with blood the ions of calcium accelerate the coagulation process however the surplus amount of calcium can provoke a burn and necrosis of tissue [9].

The aim of this study was to investigate the indexes the indexes of biocompatible pellicle hemostatic coverage *in vitro* and *in vivo* conditions.

## MATERIAL AND METHODS

The authors together with the scientific research center of chemistry and physics of polymers under the Academy of sciences of the Republic of Uzbekistan, worked out a new composite polymer material (CPM) possessing hemostatic property for the surgery of liver (30 white mature outbred male rats weighing  $198 \pm 2.7$  gr were used). (Patent No. IAP 20160273 "Bio-absorbable surgical hemostatic substance", registration from 13.06.2016, Nazirov F.G., Rashidova S.Sh., Sadykov R.A., Sarymsakov A.A., Alimov M.M., Ismailov B.A., Li Yu.B.). It presents itself as a semi-transparent flexible pellicle moderately water-soluble coverage (Picture 1). The temperature of melting is  $220^{\circ}\text{C}$ . It is stable at pH 5-7. Rapidly hydrolyzing in an alkaline environment and more steady in an acidic environment.

At the light microscopy of implant fibers of the oxidized cellulose were located evenly on the entire surface of pellicle. Methodologies of the evaluating the breaking strength, estimation of the implant's structure and the determining the adhesion power were conducted according to the Ts 05957837-28:2014 instructions and documents of the national certification system of the Republic of Uzbekistan and using the apparatus "Zwick" (Germany) and atomic-power microscope of Agilent Technologies (USA).

At the studying the blood coagulation time (BCT) in presence of hemostatic coverage on the basis of the cellulose derivatives on Lee-White test a polymer in the kind of a pellicle with the size one  $\text{cm}^2$  was placed in a test tube with one ml of venous blood. The test tube was placed in water bath at  $t - 37^{\circ}$  and incubated within two minutes, and then after every 30 sec the test tube was inclined under the angle of  $45^{\circ}$ . The time of the coagulum appearance was fixed. Control samples were investigated by analogy without adding the polymer to the whole blood.

Experimental studies of the new composition hemostatic coverage were undertaken in accordance with the Russian national standard ISO 10993-6-2011. Guideline: 30 white mature outbred male rats weighing  $198 \pm 2.7$  gr were used. Operations were performed under inhalation anesthesia (Isofluranum) with the modeling the wound of liver with an active parenchymatous bleeding. A hemostatic effect was achieved by the application of pellicle coverage (Picture 2). Morphological changes were studied in terms of 3 and 12 hours and then on the 1<sup>st</sup>, 3<sup>d</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 30<sup>th</sup> day after an operation. Macroscopic and microscopic studies were undertaken.



**Picture 1.** Sample of the Composite polymer material



**Picture 2.** Stopping the bleeding with pellicle coverage (Composite polymer material)

### Methodology of operation

Under inhalation anesthesia a supramedian laparotomy was performed with 3-4 cm lengthwise. A left hepatic lobe was withdrawn in a wound. On the surface of liver a flat wound with a diameter up to one cm and a depth up to 0.3 cm had been modeled. From the hepatic wound an active parenchymatous bleeding was marked. The pellicle coverage was applied on the bleeding surface. The control over the possible renewal of bleeding was kept by the supervision during ten minutes. A postoperative wound was sutured by interrupted sutures. Animals were withdrawn from anesthesia, and subsequently a dynamic supervision over the post-operational state was conducted.

In the fixed terms animals were withdrawn from the experiment for the estimating the macroscopic changes, and also for the intake of material for histological researches. Euthanasia was carried out according to the Provisions of ISO 10993-2 under the general anesthesia. During the experiment a macroscopic estimation of changes in an abdominal cavity was analyzed at the dissection of animals after euthanasia. For the getting ready the morphological preparations the tissue of liver was excised and fixed in 10% solution of neutral formalin. After expiration of the fixing terms bioplate was inundated by paraffin. Paraffin blocks had been manufactured. Series of sections with a thickness of three-four  $\mu\text{m}$  were made. Histological preparations were painted by hematoxylin-eosin.

For the estimating the histological changes in liver, a system of points was employed in accordance with Russian national standard ISO 10993-6-2011 where the parameters of semi-quantitative estimation of the number and distribution of cells characterizing the inflammatory process such as polymorphonuclear neutrophils, lymphocytes, plasmatic cells, macrophages, eosinophiles and multinuclear cells were taken into account. At the microscopy, the dynamics of the inflammatory reaction's development, features of the liver's parenchyma regeneration as well as the degree of the investigated implant's destruction, were estimated.

### **Ethical approval**

The review board and ethics committee of RSCS named after acad. V.Vakhidov approved the study protocol and informed consents were taken from all the participants. Experimental studies had been undertaken with the observance of the rules accepted by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS N 123), Strasbourg, 18.03.1986.

### **Statistical analysis**

The obtained results were subjected to the statistical processing with the using the standard package of Microsoft Excel 2010 software by the method of variation statistics with the estimation of indexes' values ( $M \pm m$ )

## **RESULTS AND DISCUSSION**

It was set by researches that adhesion required for medical aims had to provide the dense adherence of pellicle to the wound surface that conditioned an instantaneous hemostatic effect in the final outcome. An adhesion power of pellicle coverage made up  $7.3 \pm 0.2 \text{ N/cm}^2$ . Such a high adhesion of implant was related to its flexibility and moderate hydrophilism that strengthens this effect in the aggregate.

At the estimating the breaking strength an attention was paid to the ability of implant to hold the edges of the wound surface that provides the effect of the strengthening the tissue. It was set from the data of comparative researches that the worked out implant had a limit of the breaking strength within the limits of  $390 \pm 4.8 \text{ kGf/cm}^2$  that fully satisfied the requirements to the implants of a similar origin.

In the polymer's presence BCT on Lee-White test was shortened by as many as 2.1 times in regards of the control that made up  $2.4 \pm 0.6 \text{ min}$ . The obtained results testified to the strengthening the process of the donor blood's coagulation in vitro in the presence of implant due to the activating the factors of an external and internal way of the coagulation's hemostasis.

The results of *in vivo* researches, showed that hemostasis had started after the applying the polymeric coverage within a short time. A complete hemostasis was marked during 3-5 sec. After the supervision during 10 minutes the resumption of bleeding was not marked.

Subsequently, an activation of factors of the strengthening the hemopexis led to the forming the fibrinous pellicle on the surface of the hepatic wound. Further observations on animals showed that in an hour after an operation an implant had been preserved on the surface of liver as a white pellicle and had not been isolated from a wound surface. Bleeding signs were not marked. An abdominal cavity remained intact. In subsequent terms a substitution of pellicle coverage for fibrin without a considerable inflammatory reaction had been marked. It was paid attention to the absence of the expressed accretions in the place of the hemostatic coverage application.

The result of dissection in 3 hours after an implantation showed that there was not free liquid in the abdominal cavity, a renewal of bleeding had not been marked, fibrin pellicle was in the area of the wound. At the microscopic research it was marked an inflammatory process which was characterized in visual field by a presence of polymorphonuclear neutrophils ( $18.1 \pm 0.6$ ), lymphocytes ( $14.3 \pm 0.8$ ), plasmatic cells ( $4.4 \pm 0.4$ ), and

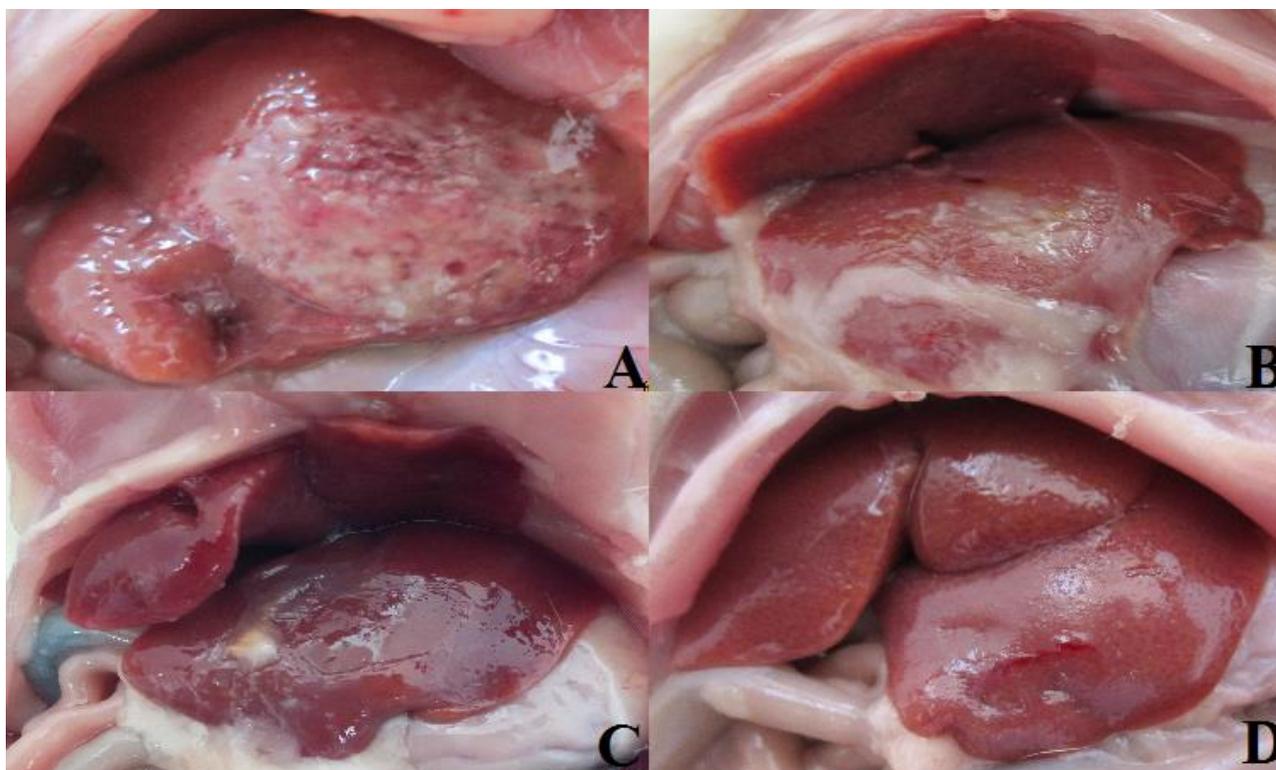
macrophages ( $2.5 \pm 0.2$ ). The pellicle fitted closely to the hepatic tissue covering the zones with signs of the stopped bleeding. The light microscopy of the wound surface of liver showed the presence of a considerable number of hemolyzed erythrocytes testifying to their appearance in the moment of trauma, and also a lot of micro-thrombus in vessels pointing to the hemostatic effect of implant.

After 12 hours, the presence of free liquid in the abdominal cavity was not revealed, a friable adhesion process with the involving the anterior abdominal wall and a dense fibrin pellicle on the wound surface were marked. The necrotic tissue with thickness  $80 \mu\text{m}$  was marked microscopically. Around the hemostatic coverage as a reaction on a foreign body an inflammatory process was marked with an increase in amount of polymorphonuclear neutrophils up to  $27.3 \pm 0.9$ , lymphocytes ( $18.8 \pm 0.9$ ), plasmatic cells ( $5.8 \pm 0.3$ ), and macrophages ( $10 \pm 0.3$ ) in visual field.

At the macroscopic research on the 1<sup>st</sup> day in an abdominal cavity a friable adhesion process with the involving the epiploon and an expressed coverage of wound surface with fibrin were noted. Microscopically subcapsularly a vacuolar dystrophy of hepatocytes with the expansion of sinusoid spaces was marked. The amount of elements of inflammation in visual field made up: polymorphonuclear neutrophils ( $29.8 \pm 0.7$ ), lymphocytes ( $13.2 \pm 0.7$ ), plasmatic cells ( $2.9 \pm 0.4$ ), and macrophages ( $7.7 \pm 0.3$ ). A vacuolar-granular dystrophy of hepatocytes and a necrotic layer of tissue with thickness up to  $120 \mu\text{m}$  were marked.

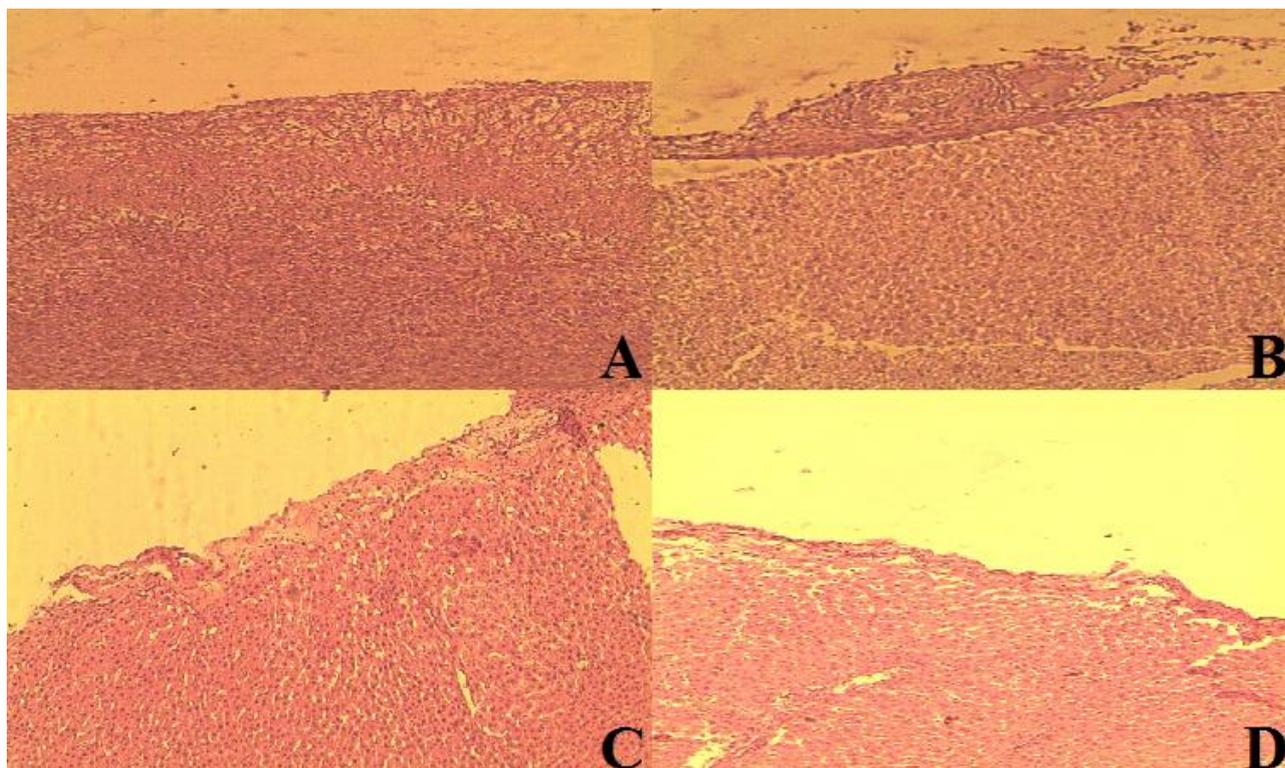
On the 3<sup>d</sup> day in an abdominal cavity a moderate adhesion process with the epiploon's areas was registered. The loops of small intestine were not engaged in a process. An insignificant decrease in fibrin pellicle was marked (Picture 3A). At the microscopy an abatement of inflammatory process was marked. The vacuolar dystrophy of hepatocytes was visualized in a subcapsular zone (Picture 4A). The thickness of the necrotic layer made up  $93 \pm 4.6 \mu\text{m}$ .

On the 7<sup>th</sup> day of the experiment in an abdominal cavity an adhesion process was preserved with the surface of liver, epiploon and xiphoid process. A decrease in fibrin pellicle was marked (Picture 3B). Microscopically the fibrosis of the hepatic capsule was marked with an abatement of inflammatory infiltrate which was expressed as a reduction in the amount of polymorphonuclear neutrophils up to  $4.7 \pm 0.6$ , lymphocytes –  $7.7 \pm 0.4$ , plasmatic cells –  $1.5 \pm 0.3$ . The thickness of the necrotic layer made up  $68 \pm 5.3 \mu\text{m}$  (Picture 4A).



**Picture 3.** Macroscopy. The 3<sup>d</sup> day after application of composite polymer material, formation of fibrin pellicle (A). The 7<sup>th</sup> day after application of composite polymer material decrease in fibrin pellicle with the forming the moderate adhesion process (B). The 14<sup>th</sup> day after application of composite polymer material, calming down of the adhesion process with the forming the pellicle coverage (C). The 30<sup>th</sup> day after application of composite polymer material, regeneration of the hepatic wound surface (D).

On the 14<sup>th</sup> day the expressed adhesion process in the abdominal cavity was not marked. A thin transparent fibrin pellicle was marked on the surface of liver (Picture 3C). Microscopically a moderate lymphoid infiltration of the hepatic capsule was marked with decrease in visual field in amount of polymorphonuclear neutrophils –  $1.3 \pm 0.4$ , lymphocytes –  $5.9 \pm 0.4$ , plasmatic cells –  $1.2 \pm 0.2$ . The necrotic layer was  $19.8 \pm 2.3 \mu\text{m}$ . The fragments of polymer were determined that testified to its resolution (Picture 4C). On the 30<sup>th</sup> day a moderate adhesion process was marked in the abdominal cavity. The wound surface of liver was of soft consistency, smooth and without a cicatricial change and signs of inflammation (Picture 3D). Microscopically a reduction in thickness of fibrotic pellicle up to  $9.5 \pm 1.0 \mu\text{m}$  was marked. A decrease in the amount of elements of inflammatory character (a rare quantity of lymphocytes) was noted. Complications and lethal outcomes during the operation and after it had not been observed (Picture 4D).



**Picture 4.** Morphology. Light microscopy. Magnification x200. Coloration Hematoxylin-Eosin. The 3<sup>d</sup> day after application of composite polymer material, vacuolar dystrophy of hepatocytes (A). The 7<sup>th</sup> day after application of composite polymer material, reduction in inflammatory process (B). The 14<sup>th</sup> day after application of composite polymer material, calming down of inflammatory process, fragments of implant (C). The 30<sup>th</sup> day after application of composite polymer material, single elements of inflammation, regenerative process (D).

Microscopically the expressed patho-histologic changes were not discovered in the hepatic tissue. A capsule of liver was not incrassate and it contained longitudinally oriented fascicles of collagen fibers. Interlobular connecting tissue was poorly developed, the signs of inflammatory infiltration and fibrosis of liver were not found. Hepatocytes were of polygonal form with a centrally located nucleus, frequently a nucleolus was determined. Quite often binuclear hepatocytes occurred. Sinusoid capillaries were of ordinary sizes. Singular erythrocytes and leucocytes were determined in a lumen. In the wall of sinusoid hemocapillaries and Disse spaces singular Kupffer cells having an intact structure were revealed at a large magnification. A moderate dilation and blood filling of sinusoid hemocapillaries, central and underlobular veins were noted in some cases. An endothelial lining was without destructive changes, in some places swelled endotheliocytes with hyperchromic nucleus were marked. The structure of cholangiol and interlobular biliary ducts was without pathological changes. And all this pointed to the fact that the studied preparation did not influence the microscopic structures of liver negatively.

From the presented data it is possible to conclude that hemostatic coverage caused a morphological reaction of liver as an inflammation and excrescence of the connecting tissue on the 1<sup>st</sup> day. But these processes calmed down quickly. A complete resolution of implant was being observed on the 7-14<sup>th</sup> days. To the 30<sup>th</sup> day

after the implantation regenerator processes in the hepatic parenchyma, especially, in the zone of lesion were observed that testified to the restoration of the tissue of liver after the applying the hemostatic coverage.

Nowadays the stopping the parenchymal bleeding from the abdominal cavity's organs remained a problem which had not entirely been solved especially when the question was about vast surfaces [10]. For the stopping such bleeding various local hemostatic substances were used in an abdominal surgery. The most widely used of them were as followed: collagen, gelatin, oxidized regenerated cellulose [9,10]. A substantial shortage of collagen and gelatin was a potential risk of pathogenic transmission because of their animal origin. At the same time the oxidized regenerated cellulose can result in an intensive inflammation of the surrounding tissues because of the low level of pH that had not been happened in the present research thanks to the selected neat correlation of ingredients of hemostatic pellicle. The following requirements were produced to the modern hemostatic substances: a high hemostatic activity at a contact with a bleeding surface, a rapid absorption of blood, a simplicity and comfort in use [1].

## CONCLUSION

As a result of joint scientific researches an optimal composition and correlation of basic ingredients of a hemostatic coverage had been worked out. A combination of three derivatives of cellulose allowed the creating the implant with hemostatic properties that meets all criteria of the solution's novelty and was the most optimal for the stopping the bleeding from the wounds of liver. Rapid enough biodegradation of the polymer along with an unexpressed inflammatory reaction made it possible to prevent the infecting related to the presence of a foreign body. Rapid formation of the fibrotic tissue in a zone of lesion allows the obtaining the stable hemostasis. The poorly expressed reaction from the side of peritoneum and surrounding organs was also marked. According to the researches' data the hemostatic coverage worked out on the basis of derivatives of cellulose possessed high mechanical parameters with a hemostatic activity and a good biocompatibility.

## DECLARATIONS

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### Authors' Contributions

All authors contributed equally to this work.

### Competing interests

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# Transluminal balloon valvotomy in the surgical treatment of mitral stenosis in pregnant women: a review

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## ABSTRACT

**Aim.** The aim of this study was to investigate current strategies in treatment of pregnant women suffering with mitral stenosis. Mitral stenosis is an obstruction of the pathway of the left ventricle flow at the mitral valve (MV) level as a result of the structural deformation of the MV apparatus, which prevents the necessary opening of the MV during the diastolic filling of the left ventricle. The most frequent lesion of valves in women with rheumatic heart disease (RHD) is MV. It remains the most common acquired valvular lesion in pregnant women and is one of the main causes of maternal death from cardiovascular diseases. According to the literature mitral stenosis (MS) is found in 75–90% of pregnant women with acquired heart defects. In addition, the incidence of fetal morbidity is positively correlated with the severity of MS: it increases from 14% in pregnant women with mild MS to 28% in people with moderate MS and 33% in women with severe mitral stenosis. The course of MS in pregnant women depends on the clinical manifestations, the degree of severity of heart failure, and the degree of rheumatic fever activity. **Conclusion.** The advantages of minimally invasive techniques during pregnancy are theoretically undeniable. The choice of the optimal method of delivery, the assessment of the fetal state of the fetus and the methods for its correction are also fundamental.

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## INTRODUCTION

Mitral stenosis is an obstruction of the pathway of the left ventricle (LV) flow at the mitral valve (MV) level as a result of the structural deformation of the mitral valve (MV) apparatus, which prevents the necessary opening of the MV during the diastolic filling of the left ventricle [1]. The most common cause of mitral stenosis (MS) is rheumatic carditis. Isolated MS is determined in 40% of patients with rheumatic heart disease, and a rheumatic history is present in approximately 60% of patients with pure MS [2-5]. The frequency of isolated MS in women and men is 2: 1 [4, 6].

India has the highest incidence of rheumatic heart disease in the South-East Asia Region, which accounts for about 40% of all cases of the disease in the world [7]. In the Republic of Uzbekistan, the prevalence of rheumatism among women aged 15-35 years living in Tashkent was 29.4%. The highest prevalence rates of rheumatic fever (RF) were found among women aged 15-19 years (39.8%). Primary morbidity was 0.6%, patients with newly diagnosed RF, including those with an already formed heart disease, 1.8% [8]. Cardiovascular diseases worsen the course of pregnancy from 1% to 3% in all pregnant women and is the cause of maternal mortality in 10-15% [9, 10]. Preterm birth in general in women with heart defects is observed in 16% of cases, and perinatal mortality in industrialized countries is 1% [11]. In low-income countries, rheumatic heart disease (RHD) accounts for approximately 90% of all cardiovascular diseases in pregnant women [9]. RHD is a disease of young people, and its effects are observed in women of reproductive age [12, 13]. For some women, the initial diagnosis is made in the antenatal or postpartum period, since women with RHD do not tolerate the effect of physiological changes of pregnancy on damaged heart valves, which leads to clinical decompensation [14, 15].

The most frequent lesion of valves in women with RHD is MV [16]. It remains the most common acquired valvular lesion in pregnant women and is one of the main causes of maternal death from cardiovascular diseases [17, 18]. Mitral stenosis is found in 75–90% of pregnant women with acquired heart defects. In addition,

the incidence of fetal morbidity is positively correlated with the severity of MS: it increases from 14% in pregnant women with mild MS to 28% in people with moderate MS and 33% in women with severe MS [19, 20].

Symptomatic mitral stenosis (MS) is associated with a higher risk of antenatal hospitalization and approximately 50% mortality, which is most common in the postpartum period [14]. During pregnancy, physiological hypervolemia creates an additional load on the heart [20, 21]. The amount of blood that must pass through the constricted mitral orifice increases. In relation to the increased volume of blood flow, the degree of narrowing of the mitral orifice is even more pronounced than before pregnancy [14, 21]. This explains the fact that the slightly pronounced so-called "silent stenosis" begin to appear only with the onset of pregnancy. According to many authors, the average degree of mitral stenosis during pregnancy can cause the same changes in hemodynamics as a high degree of stenosis in non-pregnant women [22]. The decompensation of cardiac activity that develops at the same time threatens not only the life of the mother, but also the fetus [21].

The course of MS in pregnant women depends on the clinical manifestations, the degree of severity of heart failure, and the degree of rheumatic fever activity. Factors determining the severity of MS in pregnant women [11] are: 1) AB area - openings less than 1.5 cm<sup>2</sup>; 2) pulmonary hypertension (pulmonary artery systolic pressure more than 40 mm Hg); 2) clinical signs of CH III FC (shortness of breath and tachycardia with little exertion, acrocyanosis, etc.); and 3) Atrial fibrillation - the threat of thromboembolic complications.

According to Iris et al. [23]'s work, maternal mortality occurred in 1.9% of pregnant women, ≈ 50% with severe rheumatic mitral stenosis, and 23% with significant mitral regurgitation which was complicated by heart failure during pregnancy. Consultation before pregnancy and consideration of mitral valve surgery in individual patients is important to prevent these complications.

In the absence of surgical treatment of MS before pregnancy, careful observation is recommended from the 3rd month of pregnancy and then on a monthly basis, including clinical and regular EchoCG [11, 24].

#### **Cardiac complications during pregnancy:**

- Acute left ventricular failure in the form of cardiac asthma and pulmonary edema (may occur against the background of good health);
- tachysystolic form of atrial fibrillation;
- with the development of atrial fibrillation - thromboembolic complications in a large circle of blood circulation [11].

Pregnancy with rheumatic MS is complicated in most cases with preeclampsia, hypochromic anemia, threatened abortion and fetoplacental insufficiency [15]. Pregnancy can aggravate the severity of the underlying disease, contributing to the development of: thromboembolism (occurs more often with atrial fibrillation), right ventricular HF, the occurrence of pulmonary hypertension, the development of pulmonary edema (most often occurs at 26–34 weeks of pregnancy and during labor), rhythm and conduction disturbances (40–50 % of patients are associated with the formation of a blood clot in the left atrium), a sharp increase in pressure in the left atrium (it can lead to rupture of the bronchial vein and sudden pulmonary hemorrhage). Exacerbation of rheumatic fever is also possible. Critical periods of exacerbation of rheumatic fever correspond to the first 14 weeks, 20–32 weeks of gestation and the postpartum period [25, 26, 27].

Diagnosis of a disease of the cardiovascular system during pregnancy presents certain difficulties associated with limiting diagnostic capabilities [27-29]. The most important diagnostic signs relate to the characteristics of the heart rate, the degree of increase and hypertrophy of the left atrium and both ventricles, the assessment of the severity of I and II heart sounds and the determination of the opening of the mitral valve, the prevalence of noise during systole and diastole, the magnitude and nature of amplification of this noise [30-32]. In addition to electrophysiological, functional and ultrasound methods of research, to clarify the diagnosis and/or establish the extent of damage to the cardiovascular system in pregnant women, one has to resort to a number of examinations and treatments related to the use of X-ray irradiation [11, 33]. Among practicing cardiologists and obstetrician-gynecologists there is a perception of contraindications to the use of these diagnostic methods and treatment because of their negative effects on the fetus. The effects of irradiation depend on the irradiation dose and gestational age of the fetus at the time of irradiation. If possible, these techniques should be used after the period of the main completion of organogenesis (more 12 weeks' gestation). There is no evidence of an increase in the risk of fetal malformations, fetal growth retardation syndrome (GRS) or pregnancy loss with radiation doses of less than 50 mGy. The risk of fetal malformations increases with exposure to more than 100 mGy.

**Table 1.** Evaluation of the effects on the fetus and pregnant radiation doses during various medical procedures

Technique	Radiation			
	Fetus		Mother	
Chest radiograph (straight and lateral)	<0,01 mGr	<0,01 mZv	0,1 mGr	0,1 mZv
Computed tomography (CT) of the chest	0,3 mGr	0, mZv	7 mGr	7 mZv
Coronary angiography	1,5 mGr	1,5 mZv	7 mGr	7 mZv
Transluminal balloon angioplasty (TBA) or radiofrequency ablation	3 mGr	3 mGr	15 mGr	15 mZv

During angiography in the absence of abdominal shielding, the radiation dose is 1.5 mGy, but due to the absorbing effect of the surrounding tissues, less than 20% of this dose falls on the fetus. The use of abdominal shielding in examinations and a reduction in exposure time minimize exposure dose [11]. The effect of drug therapy is higher, the greater the proportion of myocardial component in the development of chronic heart insufficiency. In severe valve dysfunction, timely treatment is the only effective treatment [34].

The possibility of therapy aimed at eliminating or weakening the etiological factor should be potentially considered in every patient with mitral disease: even with unconditional indications for surgery, etiotropic treatment will improve the patient's condition by the time of surgery, reduce its risk and achieve greater effect from the operation, than in case of unjustified refusal of such treatment. Some conditions (for example, active myocarditis of rheumatic and other etiologies) may require the transfer of the operation to a later date, prior to the suppression of the activity of the process [35-37]. When determining the indications for surgery for mitral stenosis, one should always be guided by an individual assessment of the patient's condition. The scheme of state diagnosis can only serve as a guide.

Today, surgical correction of mitral stenosis is the only way to effectively treat this disease [38]. However, traditional surgery is traumatic and can be accompanied by serious complications. The development of endovascular surgery has led to the emergence of a number of minimally invasive operations for valvular pathology. One of them is a catheter balloon mitral valvuloplasty (KBMV) [1]. The first operation to eliminate mitral stenosis was surgically performed by Souttar in 1925. The first mitral commissurotomy was performed in the early 1920s by Strjuk [39]. The introduction of instrumental mitral commissurotomy in clinical practice is associated with the names of Beiley, who in 1948 performed a series of similar interventions with good clinical results [31]. In Russia, the first such operation was performed by Bakulev in 1952 [39]. The first operation under conditions of cardiopulmonary bypass (cardiopulmonary bypass) in a pregnant woman was performed in 1959 [31].

The first in clinical practice successful prosthetics of the mitral valve with a ball prosthesis in 1960 was carried out by Starr-Edwa. Close attention to the possibility of plastic surgery on the mitral valve and their widespread introduction into the practice of most cardiac surgery centers surgeons are obliged to A. Carpentier, whose fundamental work in this direction dates back to the 1970s [31]. Closed mitral commissurotomy is performed extremely rarely and most often for health reasons in pregnant women. Contraindications for closed mitral commissurotomy are left atrioventricular opening area greater than 1.5 cm<sup>2</sup>, thrombosis of the left atrium, significant mitral regurgitation, bikomissuralnaya or heavy calcification, no commissural adhesions, concomitant severe aortic lesion and / or tricuspid valve concomitant atherosclerosis of the coronary arteries requiring surgical correction [32]. Maternal outcomes with balloon mitral valvuloplasty and open mitral commissurotomy (OMC) are the same, but fetal mortality is high at a ratio of 1: 8. Replacement MV should be carried out in severe cases; with severe cuspid calcifications and mural thrombosis, in which maternal mortality was 1–1.5% and fetal mortality 16–33% [40, 41]. Given all the above, the following indications can be formulated for the operation of mitral commissurotomy during pregnancy, taking into account the data of the clinic and instrumental methods of research [1]:

1. The lack of clinical improvement from drug treatment of heart failure for 10-12 days (increased dyspnea, congestion in the lungs, rhythm disturbance of atrial fibrillation).
2. Reduction of the heart rate and cardiovascular potential by more than 25% compared with the values of healthy pregnant women
3. Reduction in the area of the mitral valve less than 1.5 cm<sup>2</sup>, reduction or complete disappearance of "" a "" - waves in the movement of the mitral valve, the appearance of a systolic closing of the pulmonary valve cusps (according to echocardiography).
4. High pulmonary hypertension according to echocardiography and reopulmonography.

5. The presence of metabolic acidosis, respiratory alkalosis, reducing the partial tension of oxygen in the blood by 5-6% or more.

6. Violation of the efficiency of pulmonary ventilation, inconsistency of ventilation to blood flow, a sharp violation of the minute respiratory volume and oxygen utilization rate, Increase in MOU by 180-200% and more, and KIO<sub>2</sub> by 125% or less. Low tolerance threshold to physical activity (300 kgm / min or less).

The best period for heart surgery is the period from 16 to 22 weeks of gestation, because a significant improvement in regional hemodynamics requires at least 60-70 days, and respiratory function is 80-90. In addition, during the operation during these periods of pregnancy, favorable conditions are created for the development of the fetus. Surgical elimination of stenosis at a later date, despite some improvement in central and organ hemodynamics, the function of external respiration, does not fully restore compensation for blood circulation, does not eliminate fetal hypoxia. Therefore, surgical treatment of stenosis in pregnancy 30 weeks or more should be considered as a necessary measure aimed at saving the patient's life [42].

There are contraindications that need to be remembered when deciding whether to conduct a mitral commissurotomy in pregnant women:

1. The gestation period is less than 15 weeks (high probability of spontaneous abortion).
2. The gestation period is over 30 weeks (lack of time to adapt the cardiovascular system of a pregnant woman to childbirth, high risk of thromboembolic complications).
3. The patient is more than 30 years old, calcification and fibrous changes of heart valves, restenosis.
4. The activity of the rheumatic process.

After undergoing heart surgery during pregnancy, the general principles of pregnancy management in women with heart defects are applied to patients. In addition, if the operation is performed later than 30 weeks of pregnancy, careful monitoring of the hemostatic system is necessary, since during this period of pregnancy, mitral commissurotomy does not eliminate the possibility of severe thromboembolic complications [10, 43, 44].

The choice of mode of delivery is strictly individual, is directly dependent on the results of surgical correction of the defect, the gestational age at which the operation was performed and should be considered in combination with the presence or absence of obstetric pathology. The most appropriate is the method of delivery through the natural birth canal [45]. This is largely due to the peculiarity of the implementation of the MSC, when there is no direct visual control of surgical procedures. Following balloon coronary angioplasty, a method of endovascular treatment of valvular stenosis appeared - balloon valvuloplasty. At first it was used for congenital aortic stenosis and stenosis of the pulmonary valve, but now the indications have expanded significantly: balloon valvuloplasty is also used for acquired heart defects (mitral stenosis and aortic stenosis), not only rheumatic, but also caused by calcification of valves [46-49]. The use of the term "valvuloplasty" in the variant of percutaneous balloon performance is criticized, since this term is more specifically used in foreign surgical literature to describe surgical reconstructive interventions mainly in patients with mitral regurgitation [22, 50, 51]. Indeed, the method of balloon dilatation of the mitral valve more closely matches the term "balloon mitral commissurotomy" or "valvulotomy". The term "balloon valvulotomy" has already become applicable in most clinics, so we use it in this article.

Three potentially possible mechanisms underlie the therapeutic effect of balloon dilatation of the mitral valve: 1) separation of soldered commissures, 2) stretching of commissures, 3) rupture of calcifications. Since the basis of mitral stenosis in young patients is the soldering of the commissures, the gap in the stenotic zones is the main and extremely effective mechanism [44, 52]. The first results of introducing new technology into clinical practice revealed the advantages of the method, which include conducting an operation without thoracotomy, artificial lung ventilation, cardiopulmonary circulation, a short period of hospitalization and rehabilitation, low cost.

The KBMV was proposed by the professor from Japan, Inoue K. in 1984. The original development of K. Inoue was proposed to perform atrial septostomy using a special balloon in the shape of a pillow. Subsequently, this technique was adapted for dilatation of mitral, aortic and pulmonary valves. The first publication reported on 6 patients, 5 of whom had dilatation of the mitral valve, was successful, with a decrease in the average pressure drop by 53% [53]. In 1985, Lock et al. [54] from the Children's Hospital in Boston performed mitral dilatation with a balloon designed for the pulmonary valve. The authors succeeded in increasing the area of the mitral orifice from  $0.7 \pm 0.3$  to  $1.3 \pm 0.3$  cm<sup>2</sup> while simultaneously reducing the pressure drop by 54% [54]. Later, Al Zaibag et al. [55] developed a double balloon dilatation technique for the mitral valve. The results were very effective: the area of the mitral increased by more than 100%. In the modern version of the proposal, Palacios et al. [56] perform a balloon mitral valvuloplasty (BMV) with two cylinders introduced through a single

septostomy opening in the interatrial septum, which makes it possible to increase the area of the valve opening by 0.5 cm<sup>2</sup> more than when using single-balloon technology. This is due either to a larger dilatation area with two cylinders, or to an elliptical shape that cylinders take, more accurately repeating the natural opening of the mitral valve. The creation of a special balloon surface also led to more efficient dilatation [55]. Large series of studies and long-term results of BMV began to be published only in the last 30 years as a whole, they confirm the first impression that BMV in some patients is practically an alternative to a closed mitral and open mitral commissurotomy [25, 47, 48, 57]. The prognostic evaluation system proposed by Wilkins et al. [58] is now widely used for this purpose [58, 59]. Each of the 4 parameters has 4 degrees of severity of the pathological process: the mobility of the valves, subvalvular adhesions, the thickness of the valves and the degree of calcification. In general, with an echo <8, a good balloon mitral valvuloplasty (BMV) result can be assumed, and with > 12 it is unsatisfactory. In the author's version, this evaluation system was confirmed quite convincingly [59, 60], although other researchers do not note such a good correlation [61].

The average time for restenosis after a closed mitral commissurotomy is, according to some authors, about 5 years, after an open commissurotomy - 8 years [40, 62, 63]. If restenoses develop similarly after BMV and surgical intervention, then early restenoses should be characteristic of patients with initially poor indicators [52]. In fact, the term "restenosis" as applied to such patients is erroneous, more often it is simply the absence of the effect of primary dilatation. It is clear that the restenosis process after BMV does not have any specific physiological or anatomical features that distinguish it from coronary restenosis after angioplasty or aortic valvuloplasty [49]. The mechanism of restenosis, of course, includes a return to the outcome of the stretched valve components, fibrosis and calcification of the commissure and cusps zones. BMV can be performed in patients with restenosis after surgical interventions [51].

Immediate and early postoperative results were studied in patients who underwent BMV and closed or open commissurotomy [62, 64]. According to hemodynamic parameters, the numbers of complications by functional class after treatment in 48 of 49 patients, the results were almost identical [65]. These data were obtained in 2 randomized groups of patients in India. The echo forecast was within 7 and the results were quite predictable. Zerzina et al. [66] reported better BMV results compared to closed commissurotomy. However, the authors considered only the short term after the operation, used a thermodilution technique for assessing cardiac output (reassessing results for patients after BMV due to possible shunting of blood from left to right through a septostomy hole) and performed stress tests, which worsened the results in patients with recent thoracotomy.

## CONCLUSION

The advantages of minimally invasive techniques during pregnancy are theoretically undeniable. However, the MSCM has not yet received widespread distribution, since opinions on the indications for the MCCM and its effectiveness, the frequency of complications are quite contradictory [49]. The occurrence of complications in KBMV is recorded in 4.7-7.8% of patients [21]. Comparison of the catheter BMV and the closed mitral commissurotomy showed the same effectiveness of these methods [20]. Therefore, due to its high efficiency and greater safety, this X-ray surgery has been widely used in the past decade. Several major centers from Europe, Asia and Latin America published the results of successful use of catheter balloon mitral valvuloplasty in pregnant women with severe mitral stenosis [23, 38, 43, 67]. For a total of seventy-one x-ray surgical operation on the mitral valve in pregnant women described in the literature by 2002, only five fetal deaths associated with this intervention were noted [57]. And although all the authors unanimously agree that the X-ray surgical operation is highly efficient and less traumatic for mater, the remote effect of radiation on the fetus still requires its study [19, 24, 68]. Potentially, X-ray exposure during fluoroscopy may be dangerous for the unborn child. The fetus receives most of the X-ray radiation in scattered rays. During the catheter balloon mitral valvuloplasty, the total radiation dose can be estimated on average as 0.2 Rad. This is regarded as a rather insignificant, non-damaging dose. However, in the event of the need for multiple X-ray studies or the use of therapeutic sessions of radiation therapy, when the total X-ray exposure is reached in 10 rad and more, abortion is shown. Therefore, in all publications on X-ray surgical correction of mitral stenosis in pregnant women, the authors point out the need for this procedure after 20 weeks of gestation, when the development of the fetal organs and systems has been completed, the placenta is fully formed. In this case, everyone points to the need to minimize the time of fluoroscopy and the use of protective screens in the patient's small pelvis area. The authors of the above studies are unanimous in the opinion that for the fetus the risk of catheter balloon mitral

valvuloplasty is much lower than the risk of surgical methods for correcting the defect and therapeutic management of a pregnant woman suffering from mitral stenosis [68].

Some researchers, for example, [Gulraze et al. \[69\]](#) provide long-term follow-up data not exceeding seventeen years for children born to patients who have undergone catheter balloon mitral valvulotomy during pregnancy. All children had normal physical development and the absence of clinically significant anomalies. Conducting MSCM in these patients is one of the most time-consuming X-ray surgical interventions on the heart for a variety of reasons. Especially the hemodynamics characteristic of pregnancy aggravate cardiac abnormalities caused by impaired blood flow through the stenotic mitral orifice. The risk of developing acute heart failure and pulmonary edema during the preparation and conduct of the operation leaves its mark on the catheter balloon valvuloplasty technique in pregnant women [46, 51, 70-72]. Despite the existence of scientific work by domestic scientists in this field, the KBMV in pregnant women in the modern scheme of surgical treatment of mitral stenosis in cardiac surgery hospitals has not found wide application.

Also, despite certain successes achieved in recent years in the tactics of conducting pregnancy and childbirth in patients with heart defects, this problem still remains relevant. These include: the possibility, indications, timing of mitral commissurotomy during pregnancy, the size of the MO after a BMW, optimal methods for controlling central and intracardiac hemodynamics, fetal survival in such a cohort of pregnant women. The choice of the optimal method of delivery, the assessment of the fetal state of the fetus and the methods for its correction are also fundamental.

## DECLARATIONS

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### Authors' contributions

Both authors contributed equally to this review.

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The authors declare that they have no competing interests.

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# Comparative efficacy of the hemostatic implant made of the cellulose derivatives on the model of parenchymatous hemorrhage from liver

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## ABSTRACT

**Aim.** The aim was to study the comparative efficiency of the hemostatic implant made of the cellulose derivatives on a model of the parenchymatous hemorrhage from a liver. **Methods.** Experimental studies on the biocompatibility's evaluation were conducted in accordance with the Russian national standard ISO 10993-6-2011. Operations have been performed under the general anaesthetizing with the modeling the parenchymatous hemorrhage from the wound of liver. A total of 72 white mature outbred rats of both sexes weighing 196.5±2.8 g were used from which 36 ones made up group of comparison using the application hemostatic material, Sergicel® Fibrillar™. In the basic group of rodents (36) powder Heparocel in equal amounts by weight of 30 mg was applied on a wound. **Results.** From the results it is possible to come to a conclusion that the hemostatic Heparocel implant causes on the first day morphological reaction of liver as an inflammation and a spread of the connecting tissue, but these processes calm down quickly. An inflammatory reaction was less expressed than the control group. To the 30<sup>th</sup> day in the basic group after application of Heparocel biodecomposition of hemostatic implant was being marked, there were regenerator processes in the liver's parenchyma especially in the zone of lesion that testifies to renewal of liver's tissue, while in a comparison group an active degradation of the application hemostatic material began on the 30<sup>th</sup> day and an expressed adhesion process in an abdominal cavity took place. **Conclusion.** Hemostatic powder closely adjoins the liver's tissue, stops bleeding, cases of relapse of bleeding were not marked. Histological researches conducted in the dynamics of the healing showed that the wounds of liver educed that Heparocel did not cause the expressed inflammatory reaction, the zone of lesion did not exceed 150 µm, and the biodecomposition started after 14 days.

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## INTRODUCTION

Nowadays a lot of preparations and their combinations possessing the hemostatic activity at local application are well-known. All of them, however, have certain limitations. It is possible to attribute materials based on gelatin, cellulose and collagen (Spongostan, USA) and combined hemostatic materials (Tachocomb, Austria) to traditional hemostatic means. For example, preparations based on collagen possess a low hemostatic activity in humid media, badly stop hemorrhage at systemic coagulopathies and thromboclastemia, have a potential possibility of the infection, and are inactivated at autoclaving that substantially reduces their efficiency and limits a sphere of application [1].

Modern hemostatic material Tachocomb presents itself as a sponge of collagen of the horse tendons, human lyophilized fibrinogen, pig fibrin, and animal's aprotinin. Its basic defect is a presence of components of animal origin which can cause an allergic reaction [2]. Application of preparations on the basis of gelatin is connected with a high probability of the infecting in a zone of the applying the implant [3].

Implants on the basis of polymeric materials find an increasingly wider use in surgery [4]. Hemostatic preparations obtained from the polysaccharides' derivatives and, first of all, from oxidized cellulose (OC) are of a considerable interest [3, 5, 6]. OC accepted internationally after it firstly used in 1945 [7], because of its bactericidal properties, favorable biocompatibility, and overall ease of use [8]. Oxidized cellulose possesses a hemostatic action and is widely used in surgery for treatment of skin wounds, protractedly non-healing chronic ulcers, resection of kidney [9-11]. The principle of the regenerated OC's hemostatic action is in the change of pH media in anoxidosis side (pH 2.5-3.0) that creates favorable conditions for the forming the thrombocyte clot. Also anoxidosis environment in the zone of lesion contributes to the nonspecific anti-microbe activity of OC [5].

OC possesses a good biocompatibility and biodegradation, is not toxic, chemically inert, non-soluble in the water, has a fibred structure and high mechanical durability [12].

A hemostatic implant on the plants basis Ankaferd Blood Stopper (ABS, Turkey) was examined for the hemostasis from the bone tissue. An inflammatory reaction was evaluated on the amount of inflammation cells: 0-25% - weak, 25-50% - moderate, 75-100% - expressed. The factors of necrosis were considered on a qualitative sign: presence or absence. Besides the inflammatory reaction a number of newly formed osteoblasts were estimated. On the scale of Moretton for the implants of bone tissue Ankaferd was determined as an agent which does not cause an active inflammation and but assisting the tissue healing [9]. The tissue reaction caused by a hemostatic implant is one of the reliable factors of the estimating the biocompatibility of a product and conditioned by terms and character of biodegradation [13, 14]. Therefore, the aim of this study was to investigate the comparative efficiency of the hemostatic implant made of the cellulose derivatives on a model of the parenchymatous hemorrhage from a liver.

## MATERIAL AND METHODS

Hemostatic Heparin implant was worked out in SI "Republican Specialized Scientific and Practical Medical Center of Surgery named after Academician V.Vakhidov". Basic components of implants were as follow: sodium salt of carboxymethyl cellulose, oxidized cellulose and nanocellulose (Patent No. IAP 20160273) [1]. Experimental studies on the biocompatibility's evaluation were conducted in accordance with the Russian national standard ISO 10993-6-2011. Operations were performed under the general anaesthetizing with the modeling the parenchymatous hemorrhage from the wound of liver. 72 white mature outbred rats of both sexes weighing  $196.5 \pm 2.8$  g were used from which 36 ones made up a group of comparison with the using the application hemostatic material of Sergicel® Fibrillar™. In the basic group of rodents (n=36) powder Heparin in equal amounts by weight of 30 mg was applied on a wound.

Under inhalation anesthesia (Halothane) a supramedian laparotomy was performed. On the surface of liver a flat wound was formed with a diameter up to one cm, and a depth up to 0.1 cm. From the liver's wound an active parenchymatous bleeding was marked. In the comparative group hemostasis was conducted by the applying the hemostatic means Sergicel® Fibrillar™ till the stopping the bleeding completely. In the experience group a hemostatic powder Heparin was applied on the bleeding surface. An observance over the possible bleeding resumption was conducted during 10 minutes. In the fixed terms animals were taken out of an experiment for the estimating the macroscopic changes, and also for the intake of material for histological researches. Laboratorial animals were withdrawn from an experiment on the 1 -, 7 -, 14 -, 21 - and the 30<sup>th</sup> day after an operation. Euthanasia was carried out according to the Provisions of ISO 10993-2-2011 under the general anesthesia. An analysis of macroscopic picture of the abdominal cavity was made at the dissection of animals after euthanasia.

For the making the morphological preparations ready the investigated area of liver was excised and fixed in 10% solution of neutral formalin. After expiration of the fixing terms biopsate was inundated by paraffin in a shape of blocks. Series of sections with a thickness of three-four  $\mu$ m were made. Histological preparations were painted by hematoxylin and eosin. For the estimation of histological changes in liver a system of points was employed in accordance with ISO 10993-6-2011 where the parameters of semi-quantitative estimation of the number and distribution of cells characterizing the inflammatory process (polymorphonuclear neutrophils, lymphocytes, plasmatic cells, macrophages, eosinophils and multinuclear cells) were taken into account. At the microscopy the dynamics of the inflammatory reaction's development, features of the liver's parenchyma regeneration as well as the degree of the investigated implant's destruction were estimated.

Experimental studies had been undertaken with the observance of the rules accepted by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS N 123), Strasbourg, 18.03.1986. The obtained results were subjected to the statistical processing with the using the standard package of Microsoft Excel 2010 software by the method of variation statistics with the estimation of indexes' values ( $M \pm m$ ) and distinctions of the examined selections on the Student's t-criterion. Distinctions in the compared groups were considered reliable at the level of value 95% ( $P < 0.05$ ).

### Ethical approval

The review board and ethics committee of RSCS named after acad. V.Vakhidov approved the study protocol and informed consents were taken from all the participants. Study protocol as well as the study itself was approved.

## RESULTS AND DISCUSSION

An active parenchymatous bleeding was being marked in a formed wound of liver. In a basic group at the using Heprocel hemostasis started during  $34.0 \pm 2.5$  seconds (Picture 1), in a control group with the using the application hemostatic material Sergicel® Fibrillar™ the time of the stopping the bleeding made up  $69.5 \pm 5.5$  seconds (Picture 2). At the 10-minutes supervision a renewal in bleeding was observed in a basic group in two (5%) cases, and in a control – in eight (22.2%) cases.

It was noted that after application of hemostatic preparation Heprocel the wound surface due to its high hygroscopic it remained dry, while at the using the application hemostatic material Sergicel® Fibrillar™ a seepage of blood between fibers with the continuing bleeding reaching the friable hemostasis had been marked (Picture 2). The results of experimental researches on the achievement of hemostasis were presented in table 1.

The obtained results showed a reliable ( $P < 0.05$ ) distinction in the shortening the time of the hemorrhage stopping and the blood loss at the using the hemostatic implant Heprocel with respect to the indexes of Sergicel® Fibrillar™. In the fixed terms animals were subjected to euthanasia and an intake of material for the conducting the morphological estimation had been carried out. After the first day an expressed inflammatory reaction and adhesive process had not been marked in the abdominal cavity (Picture 3). A vacuolar dystrophy of hepatocytes with expansion of sinusoidal spaces was marked microscopically subcapsularly (Picture 5). The amount of elements of inflammation in visual field made up as follows: polymorphonuclear- three, lymphocytes- four, plasmatic cells- three, macrophages- two. Necrosis zone –  $500 \mu\text{m}$  (Picture 4).

On the seventh day of the experiment pellicle coverage of a pellucid-whitish color had been formed in an abdominal cavity on the front surface of liver in the area of lesion. With that a formation of the adhesive process was not found (Picture 5). Fibrosis of the liver's capsule was microscopically marked with the reducing of the inflammatory infiltrate which was expressed in the form of reduction in amount: polymorphonuclear- two, lymphocytes- three, plasmatic cells- three, macrophages- two. Necrosis zone-  $200 \mu\text{m}$ . Neovascularization - three. Signs of debris (Heprocel) (Picture 6).

On the 14<sup>th</sup> day of the experiment a moderate-mild adhesive process was marked in an abdominal cavity. There was a thin pellucid coverage on the surface of liver (Picture 7). Strengthening of regenerator processes was microscopically marked in a parenchyma, especially in a zone of lesion which was manifested as a restoration of the frame construction of liver, disappearance of edema and plethora, reduction in thickness of fibrotic pellicle of liver up to  $100 \mu\text{m}$ . A moderate lymphoid infiltration of the capsule of liver was marked with reduction in visual field in an amount: polymorphonuclear- three, lymphocytes- three, plasmatic cells- three, macrophages- two. Necrosis zone- null. Neovascularization- one, fibrosis- two, fatty infiltration - one. Signs of debris (Heprocel) were determined (Picture 8).

On the 30<sup>th</sup> day of the experiment a calming down of the adhesive process was noted in an abdominal cavity. The surface of liver was smooth and of a soft consistency, without the signs of inflammation (Picture 10). A reduction in thickness of fibrotic pellicle, decrease in amount of elements of inflammatory character (singular amount of lymphocytes) were microscopically marked. Singular elements of debris (Picture 11).



**Picture 1.** Stopping the bleeding from the wound of liver with the help of hemostatic Heprocel powder

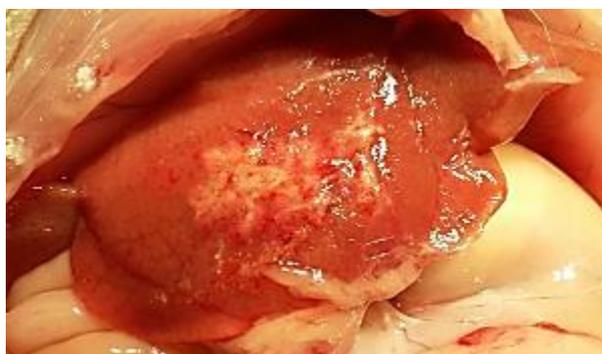


**Picture 2.** Application of hemostatic material Sergicel® Fibrillar™

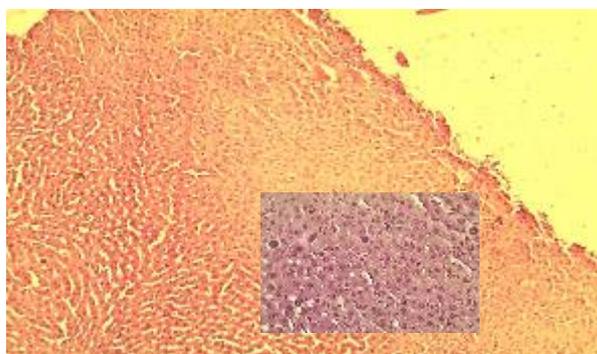
**Table 1.** Indexes of the bleeding time at the modeling the experimental parenchymatous hemorrhage from the wound of liver in rats

N <sup>o</sup>	Groups	n	Time of the hemorrhage stopping, seconds	p*	ABS	Repeated hemorrhage, %	P-value*
1	Comparative	36	69.5 ± 5.5	-	2	5,6±3.9	-
2	Basic	36	34.0 ± 2.5	P<0.001	8	22.2±7.0	P<0.05

P: an authenticity of the average values' distinctions with respect to the comparative group (accounted with the use of the Student's t-test).



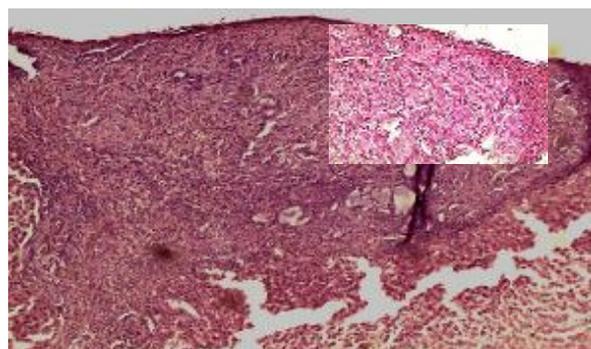
**Picture 3.** Macropicture on the first day after application of Heparcel.



**Picture 4.** Tissue of liver in an area of Heparcel application on the first day. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



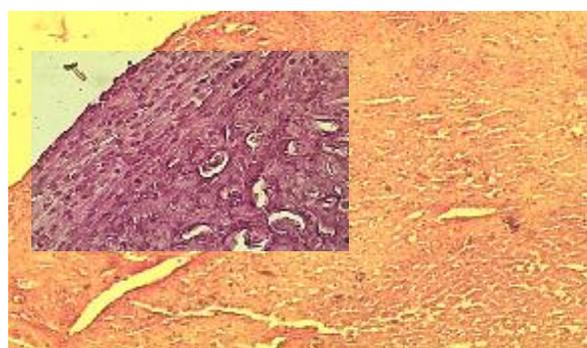
**Picture 5.** Macropicture on the seventh day after application of Heparcel. Formation of pellicle coverage.



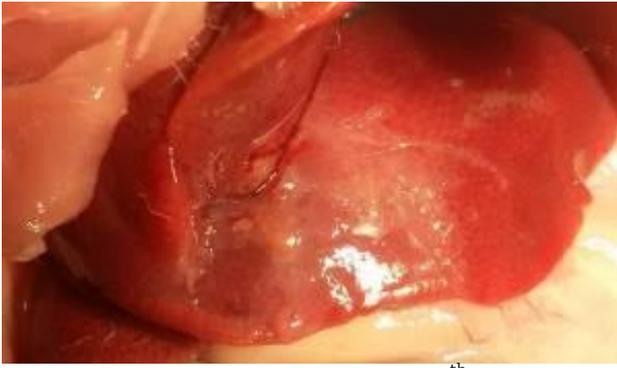
**Picture 6.** Tissue of liver in an area of Heparcel application on the seventh day. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



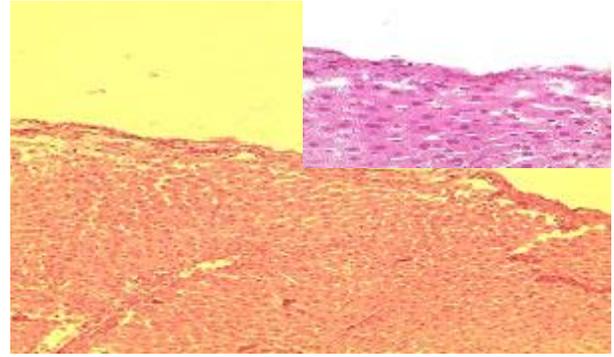
**Picture 7.** Macropicture on the 14<sup>th</sup> day. Application of Heparcel, the inning of pellicle coverage.



**Picture 8.** Tissue of liver in an area of Heparcel application on the 14<sup>th</sup> day. Absence of necrosis zone. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



**Picture 9.** Macropicture on the 30<sup>th</sup> day. Entire degradation of the hemostatic implant Heprocel.



**Picture 10.** Tissue of on the 30<sup>th</sup> day. Singular elements of Heprocel. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.

Expressed pathohistological changes in the tissue of liver were not found. The capsule of liver was not incrassated, contained the longitudinally oriented bunches of collagen fibers. Interlobular connecting tissue was developed poorly, and signs of inflammatory infiltration and fibrosis of liver were not detected. Hepatocytes of polygonal form with a centrally located nucleus, frequently a nucleolus was determined. Quite often binuclear hepatocytes occurred. Sinusoid capillaries were of ordinary sizes. Singular erythrocytes and leucocytes were determined in a lumen. In the wall of sinusoid hemocapillaries and Disse spaces singular Kupffer cells having an intact structure were revealed at a large magnification. A moderate dilation and blood filling of sinusoid hemocapillaries, central and under lobular veins were noted in some cases. An endothelial lining was without destructive changes, in some places swelled endotheliocytes with hyperchromic nucleus were marked. The structure of cholangiolandinter lobular biliary ducts was without pathological changes. And all this pointed to the fact that the studied preparation did not influence the microscopic structures of liver negatively.

In a comparison group a day after the applying the hemostatic material Sergicel® Fibrillar™ a macroscopic picture showed a hemostatic material fully saturated with blood in the wound area and a formation of adhesive process with participation of an epiploon (Picture 11). Necrosis of hepatocytes, edema of sinusoids, plethora of vessels were microscopically marked (Picture 12). The quantitative index of inflammatory process showed: polymorphonuclear- one, lymphocytes-one, plasmatic cells–one, Necrosis zone-100 µm. Neovascularization–ne, fibrosis-one. Debris-plasts.

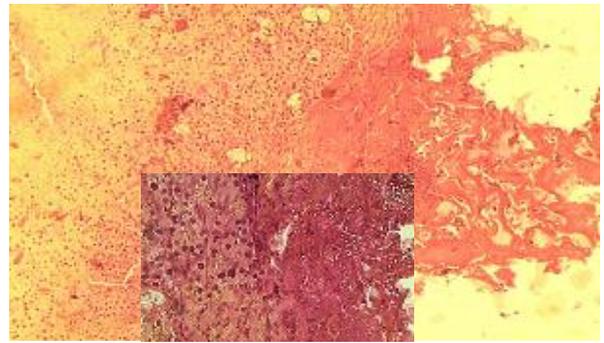
On the seventh day of the experiment on the surface of the liver's wound a preservation of structure of hemostatic material Sergicel® Fibrillar™ was noted by the formation of an adhesive process with the involving of nearby organs (Picture 13). Microscopically fibers of Sergicel® Fibrillar™ were abundantly infiltrated by neutrophil leucocytes, a preservation of hemorrhage, edema of sinusoids, and plethora were marked (Picture 14). The frame structure of liver was destroyed, and the focuses of coagulative necrosis were marked. Quantitative index of the inflammation elements: polymorphonuclear-three, lymphocytes- three, plasmatic cells - three, macrophages – one, Necrosis zone-220 µm. Neovascularization-one, fibrosis–two. Debris-plasts.

On the 14<sup>th</sup> day on the hepatic wound's surface it was marked a continuation of the preservation of hemostatic material Sergicel® Fibrillar™ fully wrapped up by an epiploon, an expressed inflammatory reaction with development of massive adhesive process took place from the side of abdominal cavity (Picture 15). An excrescence of granulation tissue rich in blood vessels with a negligible quantity of collagen fibers was microscopically marked (Picture 16). There was a strengthening of fatty infiltration. Quantitatively a composition of inflammatory elements made up: polymorphonuclear- three, lymphocytes- three, plasmatic cells - two, macrophages- one, giant cell-one, Neovascularization-two, fibrosis-two, fatty infiltrate-four. Debris-plasts.

On the 30<sup>th</sup> day of the experiment on the surface of the liver's wound a reduction in sizes of hemostatic material Sergicel® Fibrillar™ are with the preservation of adhesive process were marked (Picture 17). A quantitative reduction in elements of inflammation was also marked microscopically: polymorphonuclear-two, lymphocytes-two, plasmatic cells-one, macrophages-one. Neovascularization-one, fibrosis-two. Debris-plasts (Picture 18).



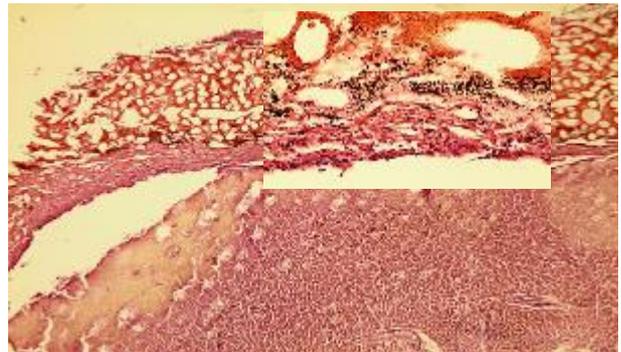
**Picture 11.** The first day. Hemostatic material Surgicel fibrillar, adhesive process with involving the epiploic and small intestine.



**Picture 12.** Tissue of liver on the first day. Application of Surgicel fibrillar, saturation with neutrophil leucocytes. Necrosis of hepatocytes, presence of debris. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.



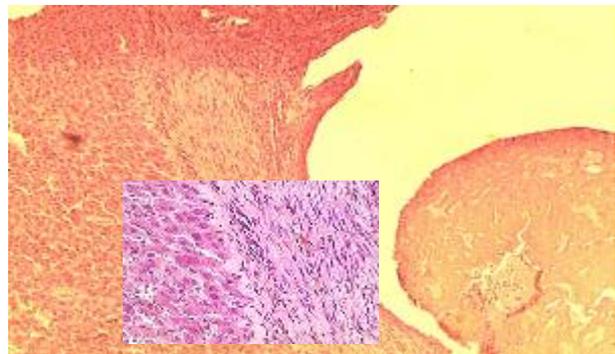
**Picture 13.** The seventh day. Hemostatic material Surgicel® Fibrillar™, formation not adhesive process with involving the small intestine and stomach.



**Picture 14.** Tissue of liver on the seventh day. Application of Surgicel® Fibrillar™. Preservation of plethora and edema of sinusoids. Presence of debris. Light microscopy. Magnification x200, x40. Coloration Hematoxylin-Eosin.



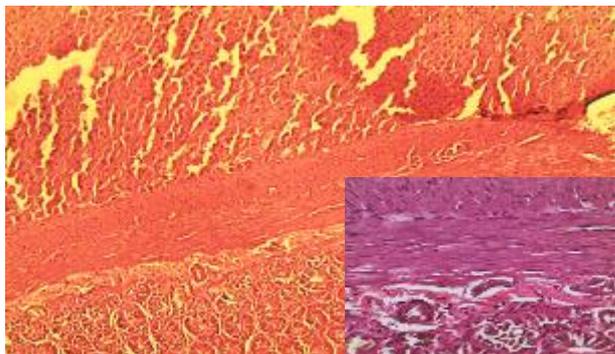
**Picture 15.** The 14<sup>th</sup> day. Hemostatic material Surgicel® Fibrillar™, formation of massive adhesive process



**Picture 16.** Tissue of liver on the 14<sup>th</sup> day. Application of Surgicel® Fibrillar™, exuberance of granulation tissue, fatty infiltration. Presence of debris. Light microscopy. Magnification x200, x40. Coloration Hematoxylin-Eosin.



**Picture 17.** The 30<sup>th</sup> day. Reduction in sizes of hemostatic material Surgicel fibrillar.



**Picture 18.** Tissue of liver on the 30<sup>th</sup> day. Fragmentation of fibers of Surgicel fibrillar. Light microscopy. Magnification x200, x400. Coloration Hematoxylin-Eosin.

At the macroscopic evaluation the structure of the application hemostatic material Sergicel® Fibrillar™ was preserved up to 30 days. Using the application hemostatic material Sergicel® Fibrillar™ promoted the development to inflammatory reaction, on the 14<sup>th</sup> day after an operation an expressed adhesive process of the abdominal cavity was marked with the involving the nearby organs which began to calm down on 30<sup>th</sup> day. On the same day an expressed biodegradation of Surgicel fibrillary began, a preparation disintegrated on fragments and was phagocytosed by macrophages, and here a number of inflammatory elements diminished.

As a result of researches it is possible to come to conclusion that a hemostatic implant Heprocel causes on the first day a morphological reaction of liver in a kind of inflammation and excrescence of the connecting tissue, but these processes calm down quickly. An inflammatory reaction is less expressed than in the group of control. To the 30<sup>th</sup> day in a basic group after application of Heprocel a biodecomposition of hemostatic implant is marked, and regenerator processes in the parenchyma of liver, especially in a zone of lesion, were observed that testifies to the restoration of the liver's tissue, while in a comparative group an active degradation of application hemostatic material began on the 30<sup>th</sup> day and an expressed adhesive process took place in an abdominal cavity.

## CONCLUSION

Thus, the results of the conducted research of the comparative efficiency showed that the created implant Heprocel comparable with a foreign analogue differed by a greater hemostatic activity, good adhesiveness to the parenchyma of liver. In in vivo researches it was marked that the acceleration of the blood coagulability's time had been by as many as two times faster than in the comparative group. From the data of our researches hemostatic powder closely adjoins the tissue of liver, stops the bleeding, cases of relapse of bleeding were not marked. Histological researches conducted in the dynamics of the healing the wounds of liver revealed that Heprocel did not cause the expressed inflammatory reaction, the zone of lesion did not exceed 150 µm, biodecomposition started after 14 days.

## DECLARATIONS

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### Authors' contributions

All authors contributed equally to this work.

### Competing interests

The authors declare that they have no competing interests.

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## Examples (at the text, blue highlighted)

Smit [1] ...; Smit and Janak [2]...; Nurai et al. [3] reported that ; ... [1], --- [2, 3], --- [3-7]. The references at the end of this document are in the preferred referencing style. Give all authors' names; do not use "et al." unless there are six authors or more. Use a space after authors' initials. Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication". Capitalize only the first word in a paper title, except for proper nouns and element symbols. For papers published in translation journals, please give the English citation first, followed by the original foreign-language citation.

## Acceptable Examples (at References section)

### For Journals:

1. Hasan V, Sri Widodo M and Semedi B. 2015. Oocyte diameter distribution and fecundity of Javaen Barb (*Systemus Orphoides*) at the start of rainy season in Lenteng River, East Java, Indonesia insurance. *J. Life Sci Biomed*, 5(2): 39-42.
2. Karen KS, Otto CM. 2007. Pregnancy in women with valvular heart disease. *Heart*. 2007 May; 93(5): 552-558.
3. Doll MA, Salazar-González RA, Bodduluri S, Hein DW. Arylamine N-acetyltransferase 2 genotype-dependent N-acetylation of isoniazid in cryopreserved human hepatocytes. *Acta Pharm Sin B*, 2017; 7(4):517-522.

### For In press manuscripts (maximum 2):

Hasan V, Sri Widodo M and Semedi B. 2015. Oocyte Diameter Distribution and Fecundity of Javaen Barb (*Systemus Orphoides*) at the Start of Rainy Season in Lenteng River, East Java, Indonesia insurance. In press.

### For symposia reports and abstracts:

Cruz EM, Almatar S, Aludul EK and Al-Yaqout A. 2000. Preliminary Studies on the Performance and Feeding Behaviour of Silver Pomfret (*Pampus argentens euphrasen*) Fingerlings fed with Commercial Feed and Reared in Fibreglass Tanks. *Asian Fisheries Society Manila, Philippine* 13: 191-199.

### For Conference:

Skinner J, Fleener B and Rinchiuso M. 2003. Examining the Relationship between Supervisors and Subordinate Feeling of Empowerment with LMX as A Possible Moderator. 24th Annual Conference for Industrial Organizational Behavior.

### For Book:

Russell, Findlay E, 1983. Snake Venom Poisoning, 163, Great Neck, NY: Scholium International. ISBN 0-87936-015-1.

### For Web Site:

Bhatti SA and Firkins JT. 2008. [http://www.ohioline.osu.edu/sc1156\\_27.html](http://www.ohioline.osu.edu/sc1156_27.html).

## Nomenclature and Abbreviations

Nomenclature should follow that given in NCBI web page and Chemical Abstracts. Standard abbreviations are preferable. If a new abbreviation is used, it should be defined at its first usage. Abbreviations should be presented in one paragraph, in the format: "term: definition". Please separate the items by ";".

E.g. ANN: artificial neural network; CFS: closed form solution; ...

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Other abbreviations and symbols should follow the recommendations on units, symbols and abbreviations: in "A guide for Biological and Medical Editors and Authors (the Royal Society of Medicine London 1977). Papers that have not been published should be cited as "unpublished". Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published". Papers that have been submitted for publication should be cited as "submitted for publication".

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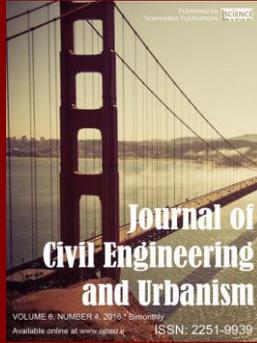
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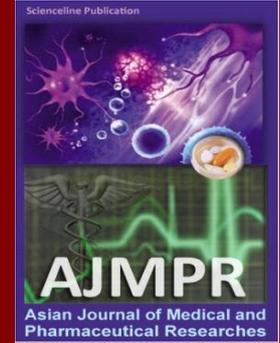
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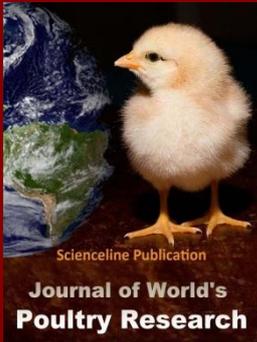
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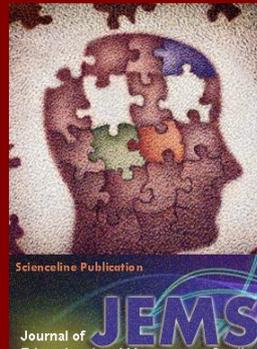
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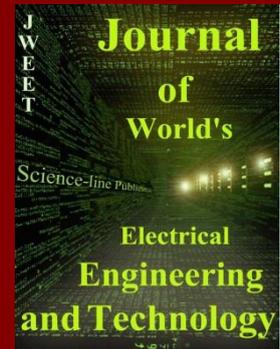
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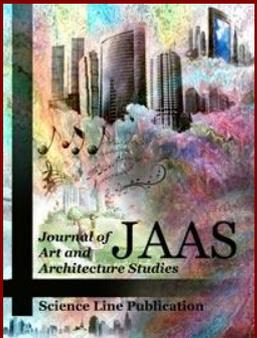
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