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Implant system for treatment of the orbital blowout fractures in the maxillofacial region using polypropylene yarn and bioactive bone cement

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Abstract

Fractures in the craniofacial region are a serious problem in terms of treatment. The most reasonable solution is the use of individual implants dedicated to a specific patient. The aim of this study was to develop the implant system specifically for treatment of the orbital floor defects of blowout fractures of maxillofacial region, using polypropylene yarn and bone cement. Three types of bone cement were used to fix the polypropylene yarn: unmodified, antibiotic-loaded and modified with nanometals. The following research was carried out: selection of cement production parameters, assessment of the curing time, measurement of polymerization temperature, an analysis of microstructure and surface topography, evaluation of wettability, measurement of microhardness, and studies of bactericidal effectiveness. The research confirms the possibility of using bone cement and polypropylene yarn for an individual implant, dedicated to the fractures treatment in the maxillofacial region. Moreover, the bactericidal properties of the proposed modifications for bone cement have been verified, hence bioactive cements are recommended for use in the case of infectious complications.

Key words

bioactive bone cement; polypropylene yarn; orbital fracture; nanometals;

1. Introduction

Orbital fractures are often injuries in the craniofacial area and constitute about 40% of all facial trauma. Not all fractures require surgical intervention, however, it is required when complications associated with the injury are life-threatening, affect health or cause great discomfort to the patient. The main indications for surgery are damage and visual disorder. The most common mechanisms of this injury are traffic accidents, beatings, sports contusions or falls from a height. The crucial problem of orbital fractures are complications, such as diplopia or enophthalmus, which require surgical approach [1-3]. In the case of an extensive fracture or crushed bone tissue, when osteosynthesis is needed, autologous grafts (harvested

from iliac crest, rib or fibula), titanium and titanium-alloy implants or high-density porous polyethylene may be used to reconstruct the orbit. When the fracture just needs to be stabilized, titanium mesh or resorbable sheeting may be applied [1,3-5].

The aim of this study was to develop an implant system for treatment of the orbital floor defects of blowout fractures using polypropylene yarn and bioactive bone cement. Polypropylene yarn, especially in the form of mesh, is widely used in medical approaches. Apart from treatment fractures, it may be used for repairing abdominal hernia, as scaffolds for cell cultures, and to conduct pelvic reconstruction [5-7]. Bone cement is a biomaterial typically used in orthopedic or traumatological treatment for stabilizing complicated fractures, fixing implants and repairing bone defects. This material is characterized by bone-like properties and a porous structure, which affects the process of osseointegration with the bone tissue and enables the creation of a stable biomechanical binding [8-10]. The purpose of using bone cement is to support yarn fixing, improve tissue binding and speed up healing. The use of polypropylene yarn, in turn, aims to give shape and stability to bone cement. The proposed system is characterized by a wide range of application possibilities, it is easy to use and also can eliminate the disadvantages of another methods of treatment such as, for example, titanium mesh, which is heavy, relatively expensive to produce and can cause soft tissue damage. Moreover, it was proposed that a modified bioactive bone cement be used, which was initially studied by the authors [11-13]. This material has bactericidal properties and, as a result, protects against infection.

2. Materials and methods

2.1 Polypropylene yarn

Polypropylene yarn, used as a scaffold for stabilizing orbital wall fractures, was supplied by Tricomed (Poland) [14]. This implant is characterized by high durability, low specific weight and it is easily malleable. Moreover, is non-toxic, chemically inactive, non-degradable and hydrophobic [14]. The polypropylene yarn used in the research is shown in Fig. 1.

Fig. 1. Polypropylene yarn produced by Tricomed (Poland)

2.2 Bone cement

Bone cement was applied to the yarn. In the research, commercially available bone cement – Cemex (Tecres, Italy) was used. Three types of cements were prepared and examined with the yarn: 1) unmodified bone cement, 2) antibiotic-loaded bone cement and 3) bone cement modified with nanometals. The modification consisted of the use of an additive: gentamicin sulfate (Sigma-Aldrich, USA) or nanometals Ag/Cu: 97:3 (MkNano, Canada). The average size of a nanoparticle was 90 nm and its purity was 99.9%. The cements were prepared following the procedure by the manufacturer's recommendation [15], but the preparation of modified cements was conducted with a preceding step, in accordance with previous study [12]. Firstly, the powder was aerated (mixing for 1 minute) and then the modifier was added to it. The mixture was mixed for 1 minute by hand with an average speed – 2 revolutions per second. The concentration of the modification had been chosen based on the preliminary results of the previous research [12,16]. The final composition of bone cement used in this work is presented in Tab. 1.

Tab. 1. The chemical composition of bone cements used for research.

The bone cements were prepared by combining the powder component with liquid in a bowl and hand-mixing at an average speed of 2 revolutions per second. Next, the obtained paste was applied on the yarn and allowed to cure for 1 hour in ambient conditions. An example of the specimens applied to the yarn are shown in Fig. 2.

Fig. 2. Specimens of the bone cement applied to the polypropylene yarn: A – bone cement modified with nanometals, B – antibiotic-loaded bone cement and C – unmodified bone cement

2.3 Selection of liquid/powder ratio

In order to choose an optimal liquid/powder ratio in terms of functionality, specimens were made (n=5) in the following ratios: 0.3, 0.35, 0.4, 0.45, 0.5. The following tests were carried out: setting time, contact angle and microhardness. Moreover, the parameters related to the potential application were evaluated.

2.4 Physical and mechanical characterization of stabilizing system

In order to evaluate the physical and mechanical properties of the examined stabilizing system, the following studies of bone cement were carried out: setting time, polymerization temperature, porosity, microhardness, a microstructure and topography analysis. The number of tested specimens was 5 (n=5). The setting time test was performed using Vicat needle apparatus (ZI-1004, India) with tip diameter of 1 mm and 400 g load in conditions simulating human body – in Ringer's solution (Fresenius Kabi, Poland) and 37°C temperature. Bone cement was considered as complete when the indentation mark on the surface was not visible. The temperature was measured continuously using a thermocouple (Czah, Poland). The samples intended for surface tests were wet ground using 2000 grit silicon carbide paper and cured for 24 h. To determine the porosity, microstructure and surface topography, a scanning electron microscope (Joel JSM-7800F, Japan) was used. The microhardness test was carried out using Vickers hardness tester (Future-Tech FM-800, Japan). The indentation press time was 10 s and the press load was 10 N. Moreover, the effectiveness of bone cement in the application aspect was examined. For this purpose, it was used to fix the polypropylene yarn to a titanium alloy plate (Ti-6Al-4V).

2.5 Bioactive characterization of stabilizing system

The bioactivity of the tested stabilizing system was determined based on the study of wettability, bacterial growth inhibition zone, and assessment of bacterial adhesion to the surface. To examine the surface hydrophilicity, an optical tensiometer (Attention Theta Life, Biolin Scientific, USA) was used. The measurements were carried out using the falling drop method. For antibacterial tests, a combination of five clinically isolated bacterial strains was used: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* (supplied by the Specialist Hospital in Kościerzyna, Poland). These strains of bacteria were selected for being the most common sources of craniofacial infections [17,18]. Each strain of bacteria was incubated separately and then added to a sterile 0.9% NaCl solution. For the study of the bacterial growth inhibition zone,

100 µl of this suspension was taken and seeded on the Mueller-Hinton agar plates. The final concentration of bacteria was 1.5×10^8 CFU ml⁻¹. The inhibition zone test consisted of placing the specimens (10 mm diameter, 2 mm thickness) on plates with the resulting bacterial suspension and incubation at 37°C. The whole experiment lasted 7 days, and the measurements of the inhibition zone were carried out after: 24, 48 and 168 hours. The bacterial growth inhibition zone was determined as an area without bacterial growth. The area of bactericidal activity was assessed by naked eye, but additionally, a biological microscope (Axio Observer D1, ZEISS, Germany) was used to analyze the bacterial medium. The experiment was performed using three specimens for each type of bone cement (n=3), but also the specimens embedded in the yarn (n=1) were checked. However, for bacterial adhesion tests 10 ml of each bacterial strain suspension was taken (inoculum - 1×10^8 CFU ml⁻¹) and added to 50 ml of the liquid medium (Tryptic Soy Bulion, Merck, Poland). The experiment was performed using one specimen for each type of bone cement (n=1). The specimens were then placed in this bacterial solution and incubated at 37°C for 30 days. A scanning electron microscope (Joel JSM-7800F, Japan) was used to assess the adhesion of bacteria to the surface and the tendency to form biofilm structures. Before the tests, all specimens were sterilized in an autoclave at 120°C for 30 min.

2.6 Statistical analysis

Statistical analysis of the data was performed using commercial software (SigmaPlot 14.0, Systat Software, USA). The Shapiro-Wilk test was used to assess normal distribution of the data. All of the results were presented as mean \pm standard deviation (SD) and were statistically analyzed using one-way analysis of variance (one-way ANOVA). Multiple comparisons versus control group between means was performed using Bonferroni t-test with statistical significance set at $P < 0.05$.

3. Results

3.1 The chosen liquid/powder ratio

The results of the adhesion to a titanium plate and setting time of the five specimens of unmodified cement are presented in Tab. 2. The evaluation of the applicability was defined in two categories: as good or as weak – based on three qualitative parameters of cement paste: consistency, ease of application and forming, as well as adhesion to the surface.

Tab. 2. Setting time and evaluation of the parameters related to the application depends on the L/P ratio for unmodified bone cement (n=5)

The ratio of liquid to powder significantly affects the quality of the obtained bone cements. Due to the method, the curing time was determined only oscillatingly, and the parameters related to the application were key. The ratio L/P=0.3 and L/P=0.5 were eliminated from further examinations, because of problematic application properties. The results of the contact angle measurements, the microhardness tests, as well as the adhesion of bone cement to the polypropylene yarn and titanium-alloy plate are presented in Tab. 3. Moreover, cements applied to the yarns are shown in Fig. 3.

Tab. 3. The results of the contact angle, microhardness tests and characteristics of the viscosity for three different L/P ratios of bone cement (mean \pm SD; n=5)

For further study, only the bone cement with a liquid/powder ratio L/P = 0.45 was used as it provided an optimal coverage of the polypropylene yarn. Moreover, the results of the contact angle measurements and microhardness test were also satisfactory.

Fig. 3. Sample specimens used in the research: A – bone cement with liquid/powder ratio L/P = 0.35; B – bone cement with liquid/powder ratio L/P = 0.4; C – bone cement with liquid/powder ratio L/P = 0.45.

3.2 Characterization of physical properties of bioactive bone cements

Setting time in conditions simulating human body and maximum polymerization temperature for the three types of bioactive bone cement are presented in Tab. 4. The curing time, due to the applied method, was determined oscillatingly. The antibiotic modification had no effect on this parameter, while the nanometals extended this time by about 2 minutes. The use of modifiers resulted in an increase of the polymerization temperature by an average of 6°C (1-9.8°C).

Tab. 4. Comparison of the tested bone cements (mean \pm SD; n=5)

3.3 Structure analysis

A comparative assessment of the topography of the obtained specimens after grinding is presented in Fig. 4. The effect of the modifications on the surface topography both before (Fig. 9) and after grinding (Fig. 4) was not observed. Moreover, porosity assessment was carried out (Tab. 5). All the cements were characterized by similar porosity.

Fig. 4. The topography analysis of bone cement surface after grinding (SEM x100): A – unmodified bone cement, B – antibiotic-loaded bone cement and C – bone cement modified with nanometals

Tab. 5. Estimated assessment of the porosity of the obtained cements (mean \pm SD; n=10)

Additionally, for the bone cement with nanometals, SEM images at high magnification were taken to confirm the deposition of nanometals and assess their propensity for agglomeration (Fig. 5).

Fig. 5. Evaluation of agglomeration of nanometals (SEM x200 and SEM x10 000)

3.4 Microhardness of bioactive bone cements

A thorough assessment of the biomechanical properties of bone cements modified with nanometals or antibiotic was carried out in the previous study [11]. In this research, for general

estimation of mechanical properties of the obtained cements, microhardness measurements were made. The results were collected in Fig. 6.

Fig. 6. Microhardness results for the bioactive bone cements: BC – unmodified bone cement, BC+A – antibiotic-loaded bone cement, BC+N – bone cement modified with nanometals (^a means significantly different from unmodified bone cement; ^b significantly different from antibiotic-loaded bone cement)

Microhardness of the cements changed due to the modification used. In the case of nanometals, it improved, however, for the antibiotic, it worsened.

3.4 Wettability of bioactive bone cements

In order to evaluate the surface properties of cements, contact angle measurements were made. The results were collected in Fig. 7.

Fig. 7. Wettability results for bioactive bone cements: BC – unmodified bone cement, BC+A – antibiotic-loaded bone cement, BC+N – bone cement modified with nanometals (^a means significantly different from unmodified bone cement; ^b significantly different from antibiotic-loaded bone cement)

The modification applied had an effect on wettability. Both modifications made it worse, as the value of the contact angle increased.

3.5 Characterization of biological properties of bioactive bone cements

In order to assess the bactericidal properties of cements, a bacterial growth inhibition zone test and assessment of bacterial adhesion to the surface was performed. The occurrence of the zone (red circle – Fig. 8) and a microscopic analysis confirm the bactericidal properties of the tested bioactive bone cement (antibiotic-loaded and modified with nanometals). However, in the case of unmodified cements, in the microscopic images live bacteria were found. The results of the bacterial tests of bone cement are shown in Fig. 8 and Tab. 6. Moreover, the entire stabilizing system (bone cement + polypropylene yarn) was tested, which is shown in Fig. 9.

Fig. 8. Bacterial growth inhibition zone for bioactive bone cements: BC – unmodified bone cement, BC+A – antibiotic-loaded bone cement, BC+N – bone cement modified with nanometals; I – after 24h, II – after 72h and III after 7 days

Tab. 6. The measurement of the bacterial growth inhibition zone for bone cements (mean \pm SD; n=3)

Fig. 9. Bacterial growth inhibition zone for stabilizing system: BC&PY – unmodified bone cement with propylene yarn, BC+A&PY – antibiotic-loaded bone cement with propylene yarn, BC+N&PY – bone cement containing nanometals with propylene yarn; I – after 24h, II – after 72h and III – after 7 days

The bacterial adhesion to the surface was also evaluated and the comparison shown in the Fig. 10.

Fig. 10. The surface of bone cements after storage in a bacterial solution (SEM x1000): A – unmodified bone cement, B – antibiotic-loaded bone cement and C – bone cement modified with nanometals

The tests have confirmed the effectiveness of the bioactive bone cement, as well as the whole stabilizing system in terms of combating bacteria. The applied modifications inhibit bacterial growth and reduce their adhesion to the surface.

4. Discussion

Orbital fractures are a common and challenging problem in maxillofacial implantology, which requires proper treatment, due to the particular construction of these areas. They mostly occur in males in their second and third decade of life. One of the main complications of orbital fractures, which is key nowadays, is face distortion. It has great aesthetic significance for the patient. There is a wide variety of available solutions (autogenous bone, autologous cartilage, titanium mesh, porous polyethylene, resorbable sheeting, glass-bioceramic), but due to their disadvantages, there is a need to search for another ones [1,18-21].

Individual implants are the answer to this problem. This is a modern method of fracture treatment or restoring bone continuity after injuries or resections. The creation of an individual implant requires the cooperation of an engineer and a doctor. Each case should be approached individually in order to get the best results. In addition to obtaining a shape which is as close as possible to an ideal one, the right choice of material is important. It must not show any toxic or allergic properties, and, at the same time, must meet a number of stringent requirements [22-24].

The use of an implant system based on polypropylene yarn and bioactive bone cements enables individual adjustment to the needs of a given patient. The proposed method seems to be better due to low cost of materials, their availability and the possibility of application for bone cavities. Moreover, it has been proposed that a modified cement that will protect against infections be used. The application of bioactive bone cement with a polypropylene yarn is to enable individual adjustment to a specific fracture, which will ensure stabilization, protection against infection after surgery, and also will remove deformities.

This work focuses on the properties of cement, so that it fulfills its task in a specific application aspect. Properties of cements based on PMMA are largely dependent on the production conditions, including liquid to powder ratio, mixing time and mixing speed [25]. Bone cement can be an element of the stabilizing system when it meets the requirements related to its intended use, i.e. ease of formation, ductility and proper adhesiveness to the surface. In preliminary studies, it was found that the key aspect in this respect is the liquid to powder ratio, and the L/P ratio of 0.45 was chosen as the most optimal one. Moreover, it is close to the manufacturer's recommendations ($P/L \approx 0.4175$) [15]. However, improper selection of this parameter resulted in the following problems: weak adhesion to the surface, heterogeneity

of the mass and its excessive fragility or fluidity. Three types of cements were tested in this research: unmodified, containing antibiotic and modified with nanometals.

The first stage of the cement assessment focused on the evaluation of their physical properties. It was found that the proposed modifications do not affect the application aspects of cement. The setting time was measured in conditions simulating the human body and was between 3 and 5 minutes. Hence, according to the obtained results, surgeon has about 3-5 min. to shape the final product to the patient's needs. After the curing process of the cement, changing the shape is more difficult, but it is still possible using additional tools. The temperature occurring during the polymerization of cements was also measured and it was about 41.5°C (36.4-46.2°C). No negative effect of this temperature on the polypropylene yarn was observed. The use of modifiers raises the polymerization temperature by an average of 6°C (1-9.8°C). The value of the measured temperature was assumed as the maximum, because it takes place inside bone cement without imitating tissue conditions (i.e. without influence of body fluids).

The structure of the obtained cements was assessed. It was found that the modification does not affect the porosity, and the average pore size was about 70 µm. Thus, the obtained cements were characterized by microporosity. However, the overall degree of porosity is not satisfactory – the structure does not show the so-called open pore structures. Porosity of biomaterials is a very important parameter because it affects the osseointegrative process and the release of the active substance from material matrix. It is assumed that macropores are best for cell deposition [26,27].

Another important parameter of bone cements is wettability. This feature allows for bone cells to be embedded into the surface and is determined by measuring the contact angle. The values of the contact angle for the obtained bone cements were different. On the one hand, this was influenced by the L/P ratio, and on the other, the modification applied. Both modifiers increased the value of the contact angle, however, the surface still showed good wettability (<90°). Such values are sufficient to ensure the osseointegrative process [28-30].

In the aspect of mechanical properties of cements, it was assumed that hardness is an important parameter, because it indirectly affects mechanical strength and brittleness. A thorough assessment of the biomechanical properties of bone cements modified with nanometals or antibiotic was carried out in the previous study [12]. In this research, the microhardness of the prepared specimens was measured. It has been found that modification with antibiotics lowers hardness, while using nanometals increase it. The obtained values of microhardness are quite similar to those from the previous study, hence comparable mechanical properties are assumed.

Bioactive cements are also expected to have bactericidal properties. The gold standard in the aspect of biomaterial modification has been the addition of antibiotics [31-33]. On the other hand, the use of nanometals was also tested in this work. This solution may be more beneficial in terms of the occurrence of a bacterial biofilm or bacterial resistance. In the literature, only a few studies were found that concerned the bactericidal activity of bone cements modified with nanometals, mainly with nanosilver. In the case of bone cements, the main conclusions suggested that only bacteriostatic properties were found and bactericidal activity was not detected [34-38]. In our research, the bacterial growth inhibition zone occurred and it oscillated around 15 mm (after 7 days). In addition, the adhesion of bacteria to the surface

after 30 days in a bacterial solution was evaluated. The entire bactericidal study confirms the effectiveness of bioactive bone cements. It is assumed that the bactericidal properties of the modifiers for bone cements may depend on the following factors: their porosity, the method of their addition to the cement matrix and the type as well as the size of the nanometals applied [11,25].

The selection of pure cement, as well as the one containing the modifiers, depends on an individual case. Research shows, that all types of cements should fulfill the task in the proposed system. In the case of infection risk, the use of bioactive cement (with an antibiotic or nanometals) is suggested. On the basis of own research and literature [11,12,34-38], the following conclusions have been drawn: antibiotics worsen the mechanical properties, while nanometals have no negative effect on them; in the case of antibiotics, there is a problem of bacterial resistance and lack of therapeutic efficacy; however, in the case of nanometals, dose selection is crucial because of their cytotoxicity. Moreover, a significant problem in both cases is to provide a long period of protection, which depends on the release of the active substance.

5. Conclusion

Nowadays, bone cements are widely used in medicine, they can be used for filling bone defects or stabilizing fractures. This paper contains research on the use of bioactive bone cement (modified with antibiotic or nanometals) to provide a system for treatment fractures in maxillofacial region. The proposed solution consisting of a polypropylene yarn with applied bactericidal cement can be used as an individual implant for a patient. In the research, the optimal liquid-to-powder ratio in bone cement dedicated for this application was selected, which is L/P = 0.45. Wettability and hardness of the cements were checked and an analysis of their topography and porosity was conducted. The obtained specimens without and with modification were characterized by: microporosity (average pores size of about 70 μm), good wettability (the contact angle below 90°) and adequate microhardness (about 20 HV). Cement obtained in conditions simulating the human body was cured for about 3-5 minutes and the maximum polymerization temperature was below 46°C. The effectiveness of bioactive cements in the bactericidal aspect was confirmed. The results of this research prove that it is possible to apply the proposed stabilizing system for specific medical cases using both unmodified and modified bone cements. Future research should consider, first of all, the assessment of osseointegration in 'in vitro' and 'in vivo' studies, and evaluation of the potential fatigue failure of the implant.

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Author contributions

Conceptualization: M.W.; methodology: M.W.; formal analysis: M.W., A.P.; investigation: M.W., D.E., A.H., A.P., G.G.; writing – original draft preparation: M.W., D.E., A.H.; writing – review and editing, M.W.; supervision: B.Ś.Ż.

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Figure Legends:

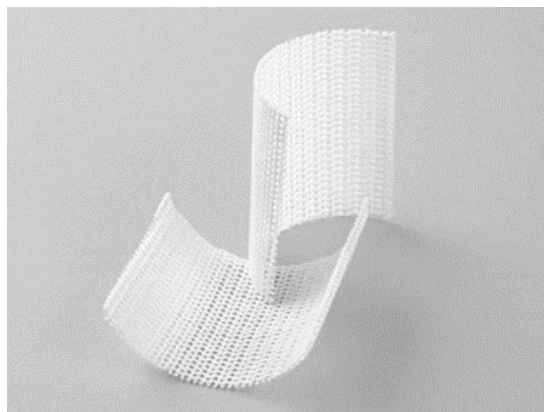


Fig. 1. Polypropylene yarn produced by Tricomed (Poland)

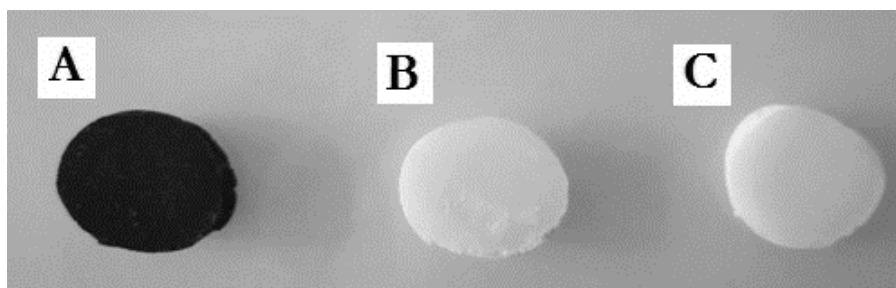


Fig. 2. Specimens of the bone cement applied to the polypropylene yarn: A – bone cement modified with nanometals, B – antibiotic-loaded bone cement and C – unmodified bone cement

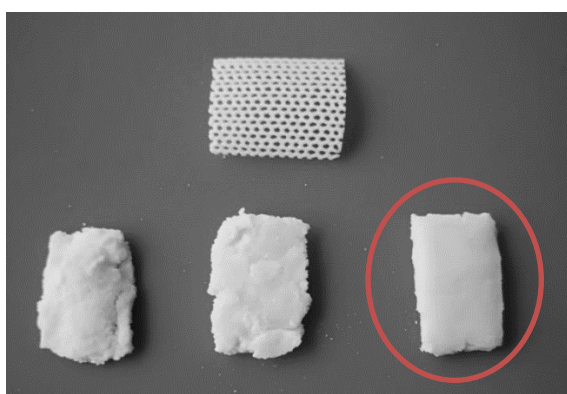


Fig. 3. Sample specimens used in the research: A – bone cement with liquid/powder ratio L/P = 0.35; B – bone cement with liquid/powder ratio L/P = 0.4; C – bone cement with liquid/powder ratio L/P = 0.45.

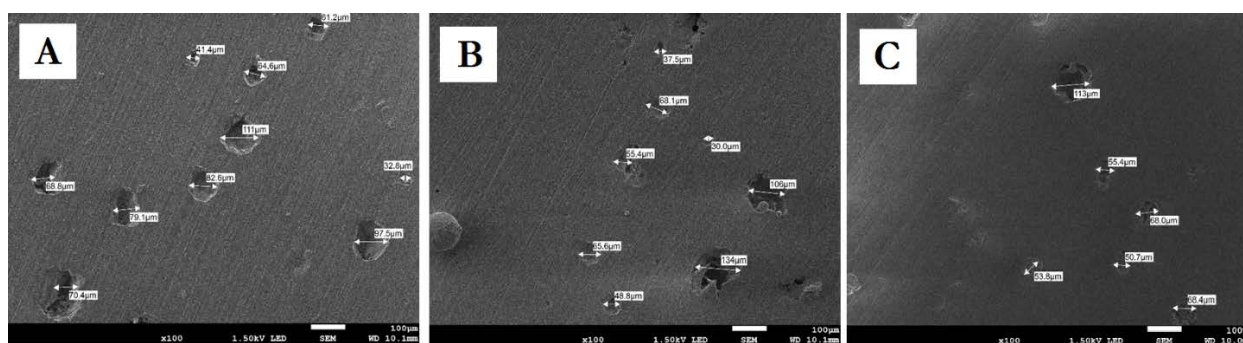


Fig. 4. The topography analysis of bone cement surface after grinding (SEM x100): A – unmodified bone cement, B – antibiotic-loaded bone cement and C – bone cement modified with nanometals

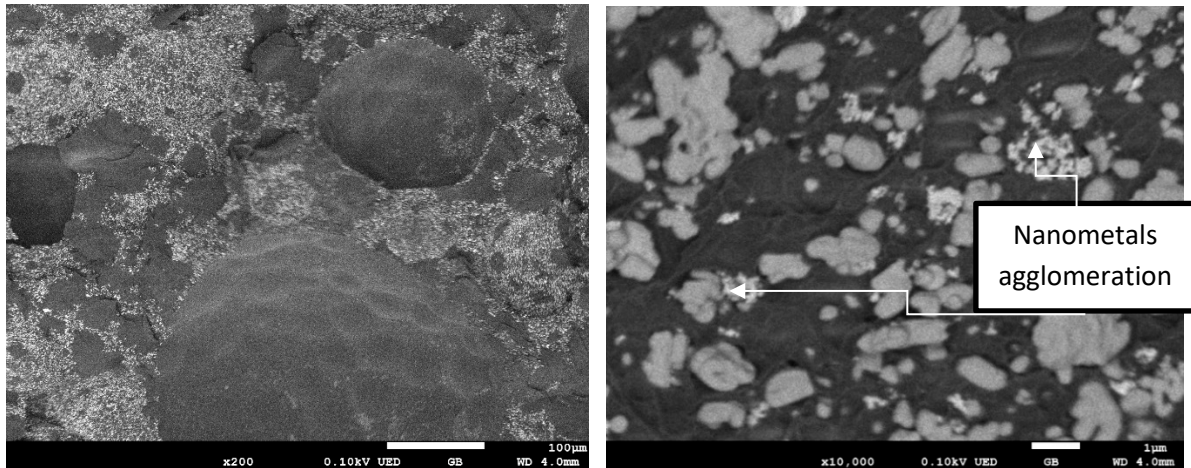


Fig. 5. Evaluation of agglomeration of nanometals (SEM x200 and SEM x10 000)

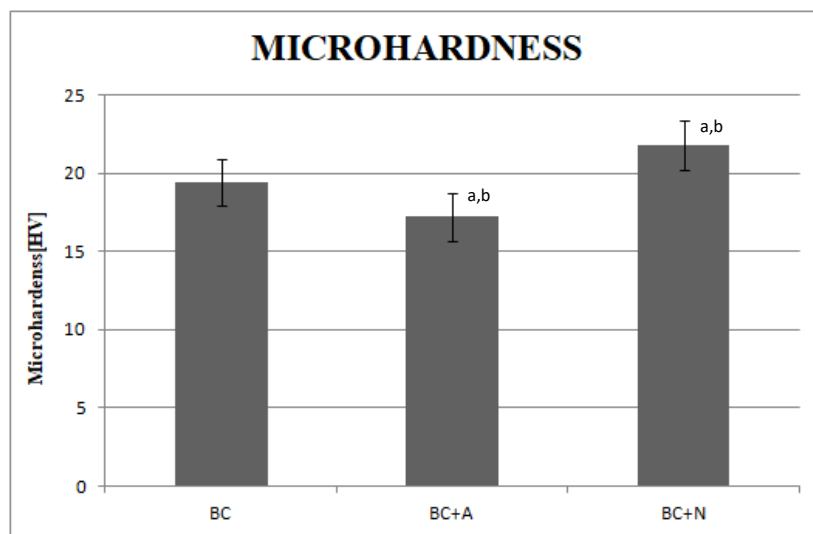


Fig. 6. Microhardness results for the bioactive bone cements: BC – unmodified bone cement, BC+A – antibiotic-loaded bone cement, BC+N – bone cement modified with nanometals (a means significantly different from unmodified bone cement; b significantly different from antibiotic-loaded bone cement)

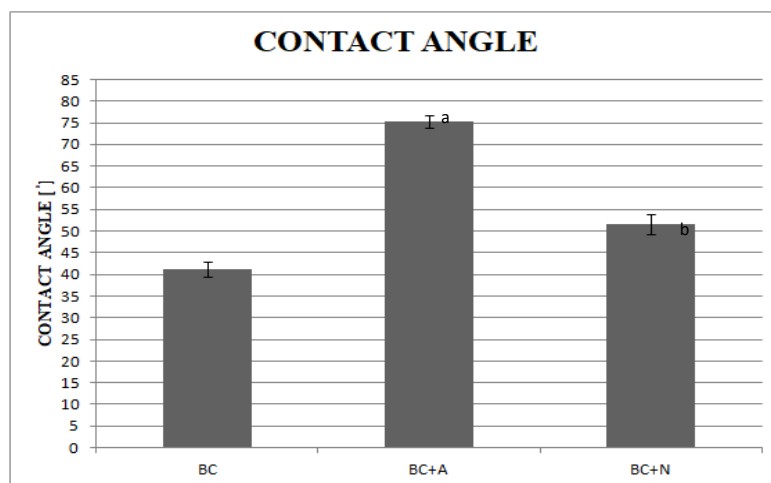


Fig. 7. Wettability results for bioactive bone cements: BC – unmodified bone cement, BC+A – antibiotic-loaded bone cement, BC+N – bone cement modified with nanometals (^a means significantly different from unmodified bone cement; ^b significantly different from antibiotic-loaded bone cement)

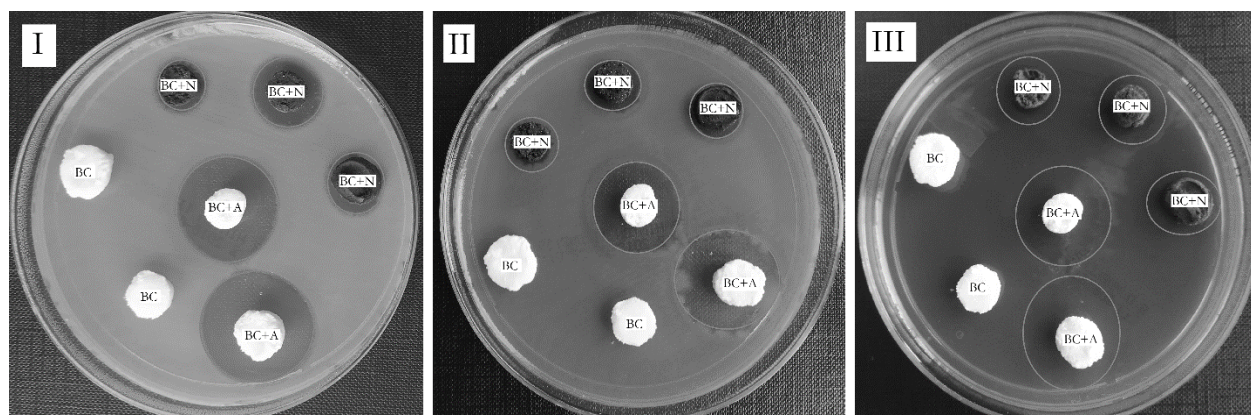


Fig. 8. Bacterial growth inhibition zone for bioactive bone cements: BC – unmodified bone cement, BC+A – antibiotic-loaded bone cement, BC+N – bone cement modified with nanometals; I – after 24h, II – after 72h and III after 7 days

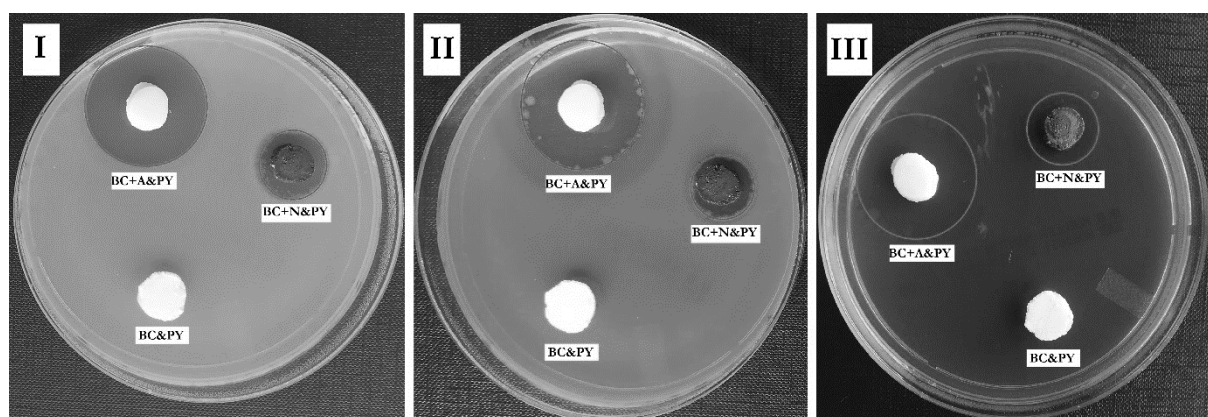


Fig. 9. Bacterial growth inhibition zone for stabilizing system: BC&PY – unmodified bone cement with propylene yarn, BC+A&PY – antibiotic-loaded bone cement with propylene yarn, BC+N&PY – bone cement containing nanometals with propylene yarn; I – after 24h, II – after 72h and III – after 7 days

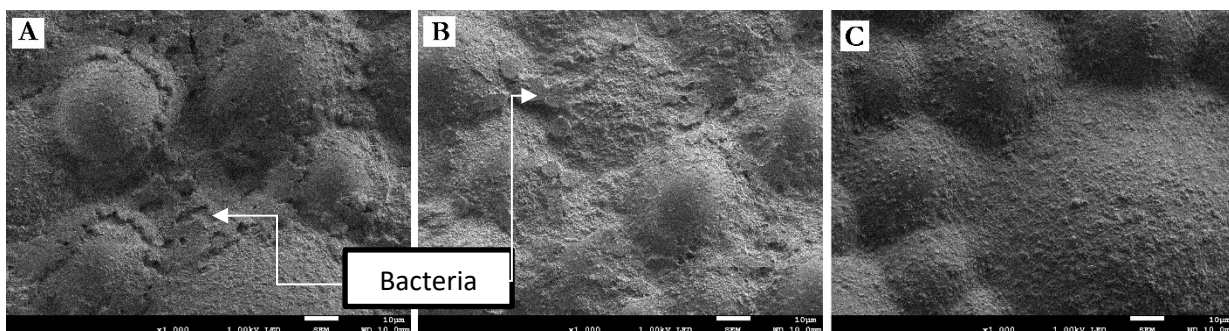


Fig. 10. The surface of bone cements after storage in a bacterial solution (SEM x1000):
 A – unmodified bone cement, B – antibiotic-loaded bone cement and C – bone cement modified with nanometals

Table Legends:

Tab. 1. The chemical composition of bone cements used for research.

| | Unmodified Bone Cement | Antibiotic-loaded bone cement | Bone Cement modified with nanometals |
|--------------------------|------------------------|-------------------------------|--------------------------------------|
| Powder component: | | | |
| Polymethyl methacrylate | 84.30% w/w | 83.46% w/w | 83.04% w/w |
| Barium sulphate | 13.00% w/w | 12.87% w/w | 12.80% w/w |
| Benzoyl peroxide | 2.70% w/w | 2.67% w/w | 2.66% w/w |
| Gentamicin sulphate | ----- | 1.00% w/w | ----- |
| Nanometals | ----- | ----- | 1.50% w/w |
| Liquid component: | | | |
| Methyl Methacrylate | 99.10% w/w | | |
| N,N-dimethyl-p-toluidine | 0.90% w/w | | |
| Hydroquinone | 75 ppm | | |

Tab. 2. Setting time and evaluation of the parameters related to the application depends on the L/P ratio for unmodified bone cement (n=5)

| No. | L/P ratio | Setting time [min] | Evaluation of application aspect |
|-----|-----------|--------------------|----------------------------------|
| 1 | 0.3 | ≈4 | Weak |
| 2 | 0.35 | ≈2 | Weak |
| 3 | 0.4 | ≈3 | Good |
| 4 | 0.45 | ≈3 | Good |
| 5 | 0.5 | ≈3,5 | Weak |

Tab. 3. The results of the contact angle, microhardness tests and characteristics of the viscosity for three different L/P ratios of bone cement (mean \pm SD; n=5)

| | Unmodified bone cement | | |
|--|--|--|--|
| | L/P = 0.35 | L/P= 0.40 | L/P= 0.45 |
| Contact angle [°] | 96.5 \pm 5.6;* | 27.38 \pm 2.2;* | 40.5 \pm 1.8;* |
| Microhardness [HV] | 14.2 \pm 1.3;* | 24 \pm 0.7;* | 17.6 \pm 0.9;* |
| Characteristics of the viscosity and adheres to the surface | The bone cement quickly gelled, did not cover the plate and yarn well, it was brittle and fragile. | The bone cement covered better than cement with a concentration of 0.35, however, it solidified quickly and homogeneous covering of the yarn and plate was not possible. | The bone cement was quite viscid, it allowed homogeneous covering and penetration into the yarn. |
| * significantly different between groups (ANOVA p<0.05) | | | |

Tab. 4. Comparison of the tested bone cements (mean \pm SD; n=5)

| | The unmodified bone cement | The bone cement modified with addition of antibiotics | The bone cement modified with addition of nanometals |
|--|----------------------------|---|--|
| Setting time in condition simulate the human body [min] | \approx 3 | \approx 3 | \approx 5 |
| Maximum polymerization temperature [°C] | 38.6 \pm 2.2 | 44.2 \pm 1.5; ^a | 44.0 \pm 2.2; ^a |
| ^a significantly different from unmodified bone cement (ANOVA p<0.05) | | | |
| ^b significantly different from antibiotic-loaded bone cement (ANOVA p<0.05) | | | |

Tab. 5. Estimated assessment of the porosity of the obtained cements (mean \pm SD; n=10)

| | The unmodified bone cement | The bone cement modified with addition of antibiotics | The bone cement modified with addition of nanometals |
|---|----------------------------|---|--|
| Average pores size [µm] | 72.5 \pm 24.5 | 68.78 \pm 35.3 | 71.72 \pm 23.2 |
| There are no statistically significant differences. | | | |



Tab. 6. The measurement of the bacterial growth inhibition zone for bone cements (mean \pm SD; n=3)

| | Bacterial growth inhibition zone – diameter [mm] | | |
|--|--|---|--|
| | The unmodified bone cement | The bone cement modified with addition of antibiotics | The bone cement modified with addition of nanometals |
| 24h | 0 | 27.4 \pm 2.1 ^{a,b} | 14.8 \pm 1.8 ^{a,b} |
| 72h | 0 | 22.6 \pm 1.6 ^{a,b} | 12.1 \pm 1.6 ^{a,b} |
| 7 days | 0 | 25.5 \pm 1.8 ^{a,b} | 15.3 \pm 1.6 ^{a,b} |
| ^a significantly different from unmodified bone cement (ANOVA p<0.05) | | | |
| ^b significantly different from antibiotic-loaded bone cement (ANOVA p<0.05) | | | |