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Antibacterial evaluation of bioactive modifiers of bone cements: antibiotics, nanometals and chitosan

Ocena antybakteryjna bioaktywnych modyfikacji cementów kostnych: antybiotyków, nanometali i chitozanu

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STRESZCZENIE

Współczesne biomateriały dodatkowo poza swoimi podstawowymi zadaniami mogą posłużyć jako nośniki substancji aktywnych. Uwalnianie bioaktywnych cząstek pozwala na lokalne zwalczanie infekcji oraz jej prewencję.

Cementy kostne także poza swoimi głównymi aplikacjami w ortopedii mogą posłużyć aby dostarczać lokalnie substancje aktywne. Obecnie, tylko antybiotyki są rutynowo stosowane jako modyfikatory dla cementów kostnych. Bioaktywne cementy kostne stanowią ciągle rozwijającą się grupę biomateriałów.

W tej pracy przeprowadzono analizę efektywności bakteriobójczej wybranych bioaktywnych substancji użytych do modyfikacji cementów kostnych. Próbki z cementu kostnego na bazie PMMA zawierające dodatkowo antybiotyki (gentamycynę i ciprofloxacin), nanometale (nanosrebro i nanomiedź) oraz chitozan zostały przygotowane. Następnie próbki te poddano testom w aspekcie zwalczania bakterii. Przeprowadzono pomiar strefy zahamowania wzrostu bakteryjnego dla kombinacji trzech szpitalnych szczepów bakterii: *Staphylococcus aureus, Pseudomonas aeruginosa* i *Escherichia coli*. Potwierdzono skuteczność cementów kostnych modyfikowanych antybiotykami oraz nanometalami. Zakłada się, że możliwe jest wykorzystanie cementów kostnych jako nośników substancji aktywnych.

ABSTRACT

Modern biomaterials in addition to their basic tasks, can serve as carriers of active substance. The release of a bioactive particles allows to locally fight infection or its prevent it.

Bone cements additionally to their basic applications in orthopedic surgery can also serve to deliver locally active substances. Currently, only antibiotics are routinely used as modifiers for bone cements. Bioactive bone cements constitute a constantly growing group of biomaterials.

In this work, analysis of the bactericidal effectiveness of selected bioactive substances used as cement modifiers was performed. Specimens of PMMA bone cement with the following additives were prepared: antibiotics (gentamicin and ciprofloxacin), nanometals (nanosilver and nanocopper) and chitosan. Then this specimens were tested in the aspect of combating bacteria. The bacterial growth inhibition zone for a medium composed of three hospital strains: *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* was measured. The efficacy of bone cements containing antibiotics or nanometals has been confirmed. It is assumed that the use of bone cements as carriers of active substances is possible.

Słowa kluczowe: *cement kostny; aktywność antybakteryjna; modyfikacja bioaktywności.*

Key words*: bone cement; antibacterial activity; bioactive modification.*

Antibacterial evaluation of bioactive modifiers of bone cements: antibiotics, nanometals and chitosan

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Abstract

Modern biomaterials in addition to their basic tasks, can serve as carriers of active substance. The release of a bioactive particles allows to locally fight infection or its prevent it.

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Key words

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1. Introduction

Active biomaterials are an important role in the aspect of modern medicine. They are expected to locally release the active substance (mainly antibiotics). This allows the prevention of potential infection as well as its therapy. Therefore the use of such a biomaterials reduces the risk of postoperative complications [1-3].

Bioactive bone cements have been particularly interesting in recent times. Typically, cements are used in orthopedic and traumatological treatment for: stabilizing complicated fractures, fixing implant or generally repair bone defect [4,5]. These materials based on PMMA /poly(methyl methacrylate)/ are characterized by self-polymerization and a curing process, which results in a porous structure with bone-like properties. This structure on one hand provides an osseointegrative process, and on the other allows the gradual release of the active substance [6,7]. Nowadays, only antibiotic-loaded bone cements (i.a. with gentamicin, cefazolin or ciprofloxacin) are commercially used. However, experimentally were tested: metal ions (e.g. Ag, Cu, Zn), particles of chitosan and nanoparticles of silver or gold [8-10].

The aim of these studies was to create modified bone cements and compare the bactericidal effectiveness of selected bioactive additives used as its modification. Typical tests of bacterial growth inhibition zone were performed for a combination of three popular orthopedic strains of hospital bacteria.

2. Materials and methods

2.1 Cement preparation

In this work, commercially available PMMA bone cements Cemex (Tecres, Italy) have been modified using bioactive additives: 1) antibiotics: gentamicin (Sigma Aldrich, Germany) and ciprofloxacin (Sigma Aldrich, Germany), 2) nanometals: silver nanoparticles – 50 nm (MkNano, Canada) and copper nanoparticles – 30 nm (MkNano, Canada) and 3) medium molecular weight chitosan (Sigma Aldrich, Germany). The modification was carried out in accordance with previous studies [11,12]. The additives were added to powder before preparing the cement and hand-mixing. Next the cements were prepared following the procedure by the manufacturer's recommendation. Then this obtained paste was placed into molds to ensure the required shape and allowed to cure for 1 hour in ambient conditions. The additives concentration was selected based on literature and previous studies [11,13-15]. The final chemical composition of cements is presented in the Tab. 1.

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

	Unmodified Bone Cement	**Bone cement modified with antibiotic**	**Bone cement modified with nanometals**	**Bone cement modified with chitosan**
Powder component:				
Polymethyl methacrylate	84.30% w/w	83.04% w/w	80.09% w/w	81.77% w/w
Barium sulphate	13.00% w/w	12.80% w/w	12.35% w/w	12.61% w/w
Benzoyl peroxide	2.70% w/w	2.66% w/w	2.56% w/w	2.62% w/w
Bioactive Additives	----------	1.50% w/w	5.0% w/w	3.00% w/w
Liquid component:				
Methyl Methacrylate	99.10% w/w			
N,N-dimethyl-p-toludine	0.90% w/w			
Hydroquinone	75 ppm			

2.2 Antibacterial evaluation

To determine the bactericidal properties of bone cements, the bacterial growth inhibition zone test following method by Bauer-Kirby et al. (1966) and Brown et al. (1975) was used [16-18]. For research three clinical isolated bacterial strains were taken: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (supplied by Specialist Hospital in Kościerzyna, Poland). These strains were selected based on the frequency of infection in orthopedic area [19].

Each bacterial strain was incubated separately and then added to the bacterial suspension. Next a 100 µl of this suspension was taken and seeded on the Mueller-Hinton agar plates. The final bacterial inoculum had value $1.5x10^8$CFU ml^{-1}. The experiment consisted of placing the specimens – modified cement disk (10 mm in diameter and 2 mm thickness) in the bacteria plates and incubation at 37°C. Before the test, the specimens were sterilized in an autoclave at 120°C for 1 hour.

The bacterial growth inhibition zone was determined as an area without bacterial growth and was assessed by naked eye. The whole experiment lasted 7 days long, and the measurement

of inhibition zone were carried out after: 24, 72 and 160 hours. The area of inhibition zone was measured using a ruler (±1mm) and additionally, the bacterial medium was checked using a biological microscope (Axio Observer D1, ZEISS, Germany).

2.5 Statistical analysis

Statistical analysis of the data was performed using commercial software (SPSS Statistics 24, IBM Corporation, USA). All of the results were presented as mean ± standard deviation (SD).

3. Results

The prepared specimens of modified bone cement were placed in a bacterial medium and their effect on bacteria was checked. The bacterial growth inhibition zone was observed in the cements modified with antibiotics and nanometals. The bacterial medium was additionally checked using a biological microscope and the absence of live bacteria was found. In the case of unmodified cement or modified with chitosan, no bactericidal effectiveness was found. The results were collected in Tab. 2. Moreover, to illustrate the effectiveness of bioactive cements, sample photos have been added (Fig. 1-3).

Tab. 2. Measurement of bacterial growth inhibition zone of tested specimens (n=5; ±SD):

Specimen	The diameter of bacterial growth inhibition zone* [mm]		
	24h	48h	7 days
Bone cement /BC/	----------	----------	----------
Bone cement modified with gentamicin /BC+A:G/	26.6 ± 1.4	26.8 ± 2.1	25.2 ± 2.4
Bone cement modified with ciprofloxacin /BC+A:C/	31.2 ± 1.9	32.3 ± 2.1	32.2 ± 2.6
Bone cement modified with nanosilver /BC+NP:Ag/	12.8 ± 2.8	13.2 ± 3.0	14.7 ± 3.2
Bone cement modified with nanocopper /BC+NP:Cu/	21.5 ± 1.6	19.3 ± 2.2	20.1 ± 2.5
Bone cement modified with chitosan /BC+Chit/	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

*the size of the specimens were 10 mm

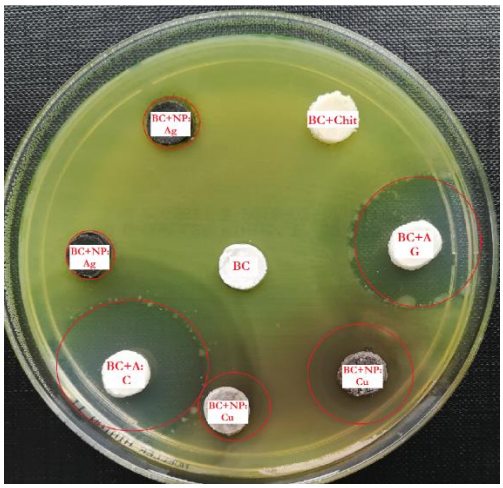

Fig. 1. Comparison of the bacterial growth inhibition zone for the tested specimens after 24 h; red circle – visible growth inhibition zone

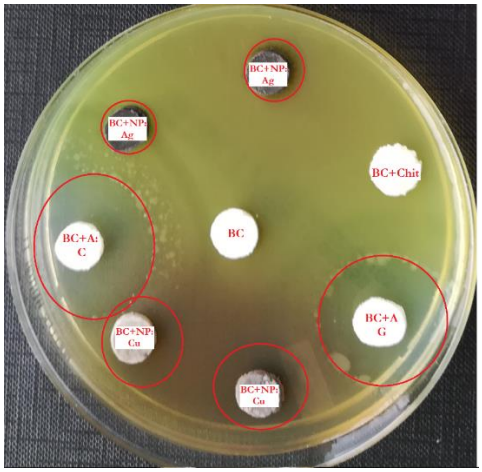

Fig. 2. Comparison of the bacterial growth inhibition zone for the tested specimens after 48 h; red circle – visible growth inhibition zone

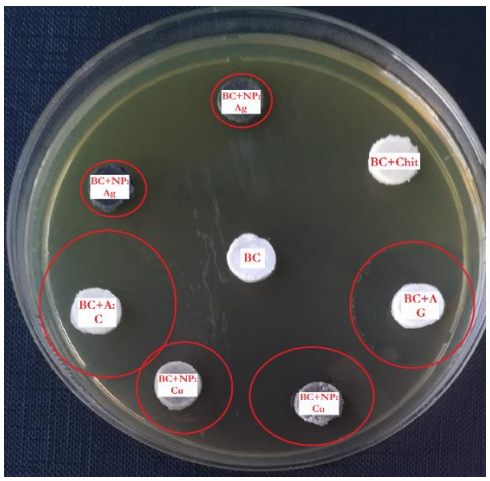

Fig. 3. Comparison of the bacterial growth inhibition zone for the tested specimens after 7 days; red circle – visible growth inhibition zone

4. Discussion

Bone cements due to their porous structure with a system of channels and corridors can be used for the local release of active substances. The particles of the substances are placed in the pores of the cement before the polymerization stage and then are gradual released as a result of the body fluids flow. Therefore, the structure of cement, and above all its porosity, has the greatest impact on the effectiveness of substance release. On the other hand, the release also affects: the amount of substances used, its form and particles size.

Generally, in the case of bioactive biomaterials the gold standard is the addition of antibiotics. However, this method becomes problematic due to the growing problem of antibiotic resistance, mutations and the formation of biofilm by bacteria. Biofilm is a specific structure, which is characterized by production of a slimy extracellular matrix. Then bacteria are protected from external factors, and what is particularly important antibiotic therapy. Therefore, currently other active substances are sought. For this potential substances the following features are expected: a broad spectrum of activity, a lack of resistance, an ability to combat biofilm and a long therapeutic period.

In this work, 5 types of bioactive PMMA bone cements modified using: gentamicin, ciprofloxacin, nanosilver, nanocopper and chitosan have been prepared. Then they were subjected to bactericidal effectiveness studies. A typical tests of the bacterial growth inhibition zone was performed. The bacterial medium consisted of three hospital strains of popular orthopedic bacteria. The bactericidal effectiveness of bone cements modified with antibiotics and nanometals has been confirmed. The largest zone of bacterial inhibition was observed for the antibiotic: ciprofloxacin. However, the smallest for nanosilver. Moreover, the lack of efficiency was found for chitosan. The experiment lasted 7 days and both antibiotic-loaded cements and cement modified with nanometals maintained their activity during this period of time.

5. Conclusion

Modified bone cements may have bactericidal properties and may be used for local treatment of infection or prevention. In this studies confirmed that effective bioactive additives for modification of bone cements may be: antibiotics (gentamicin or ciprofloxacin) or nanometals (nanosilver or nanocopper). In contrast, the effectiveness of chitosan is excluded.

Therefore, as a results of the growing problem of bacterial resistance and post-operative infections, it is crucial to look for new bioactive modification for biomaterials, e.g., such as bone cements.

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