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ORIGINAL ARTICLE

Balancing strength and sterility: An *in vitro* assessment of the mechanical and antibacterial properties of bone cement loaded with various antibiotics

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First-line treatments in the therapy of orthopedic infections include surgical debridement, local adjuvant agents, as well as systemic and local antibiotic applications.[1] There are various techniques for the application of local antibiotic treatments, which is one of the treatments used for the therapy of implant-induced or primary bone infections. Antibiotic-loaded bone cement applications are prominent among local treatments.[2] Since the use of antibiotic-loaded bone cement in total joint replacement surgery has increased and became widespread, deep infections have been treated more effectively.[2,3]

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ABSTRACT

Objectives: This study aims to determine the antimicrobial efficacy and mechanical strength of antibiotic-loaded cement samples in which daptomycin, vancomycin, teicoplanin, meropenem and piperacillin-tazobactam agents were mixed at different doses and to investigate the differences between agents and between doses.

Materials and methods: We prepared standardized bone cement samples incorporating vancomycin, teicoplanin, daptomycin, piperacillin-tazobactam, and meropenem at concentrations ranging from 0.625 to 15%. Samples underwent six-week phosphate-buffered saline immersion simulating physiological conditions. Mechanical testing employed four-point bending analysis following ISO 5833 standards. Antimicrobial efficacy was assessed using liquid microdilution against *Staphylococcus aureus* and *Pseudomonas aeruginosa* over 21 days.

Results: All antibiotics demonstrated strong negative correlation between concentration and mechanical strength ($r=-0.883$ to -0.914, $p<0.001$). Critical threshold emerged at 5% antibiotic concentration, above which mechanical integrity was significantly compromised. Daptomycin and meropenem maintained superior mechanical properties at effective antimicrobial concentrations. At 2-g doses, daptomycin achieved significantly higher strength than piperacillin-tazobactam and teicoplanin ($p=0.021$). All antibiotics except low-dose piperacillin-tazobactam maintained complete antimicrobial efficacy throughout 21-day testing. Electron microscopy revealed increased porosity, which correlated with reduced mechanical strength, particularly in piperacillin-tazobactam and teicoplanin groups.

Conclusion: Antibiotic selection significantly impacts both mechanical and antimicrobial properties of bone cement. Daptomycin and meropenem offer optimal balance of antimicrobial efficacy and mechanical integrity. These findings provide evidence-based guidelines for antibiotic selection in resistant infection management while maintaining structural requirements.

Keywords: Bone cements with antibiotics, infected bone, mechanical strength.

Antibiotic-loaded bone cements are an important treatment method for the treatment of a persistent infection, and they are involved in both local antibiotic release and load bearing. Therefore, the amount of antibiotic to be added to the cement is critical in terms of mechanical strength.[1] However, issues related to the role of bone cements in preventing infection, the type of antibiotic to be used, antibiotic resistance, and the effectiveness and costs of antibiotics are still being investigated.

Loading antibiotics in bone cement has many proven advantages. Some of these advantages include low systemic toxicity,[4] high local antibiotic release,[5] and minimal local tissue toxicity since it affects a limited area.[5,6]

Under current conditions, resistant bacterial infections are very common and commercially available antibiotic-loaded cements do not provide sufficient efficacy against them. In particular, commercially used antibiotic-loaded cements contain gentamicin and tobramycin, which are ineffective against resistant microorganisms. Appropriate antibiotics that would be effective against resistant microorganisms can be added to the bone cement using the hand mixing method, thereby achieving the desired antibacterial effect.

Mixing an antibiotic in bone cement such as vancomycin or teicoplanin, which can act on antibiotic-resistant strains, is considered to be important for more successful treatment.[7] However, the exact effects of daptomycin and meropenem, which are reported to be frequently used for resistant infections in the literature, and piperacillin-tazobactam, which is effective against Gram-negative bacteria and especially Pseudomonas, are not known for local applications. Furthermore, the interaction of these antibiotic agents with bone cement and the mechanical adequacy of the resulting mixtures still remain unknown.

In the present study, we aimed to determine the antimicrobial efficacy and mechanical strength of antibiotic-loaded cement samples in which daptomycin, vancomycin, teicoplanin, meropenem and piperacillin-tazobactam agents were mixed at different doses and to investigate the differences between agents and between doses.

MATERIALS AND METHODS

A total of 21 units of Biomet® Bone Cement (Biomet Orthopedics Switzerland GmbH, Switzerland) brand 40 g radiopaque bone cement were used in this *in vitro* study. The samples were prepared under sterile operating room conditions at a temperature of 19±2°C, for mechanical testing and antibiotic release experiments. As this is a laboratory study and does not involve human or animal subjects, ethical approval is not required.

A Kern PCB 3500-2 (Schramberg, Germany) precision balance was used to measure the amount of cements and antibiotics. Separate measuring cups were used for each cement and antibiotic to prevent cross-contamination between agents. The measuring cups were placed on a precision scale and tared and, then, the scale was zeroed before weight measurements were made for all agents.

To perform the mechanical bending tests, the samples were prepared in the form of rectangular prisms with the dimensions of 80×10×4 mm, according to the ISO 5833 Regulation. An NC-Z CNC machine was used to prepare the molds, which minimized the margin of error through computerized systems; steel casting molds were fabricated in which 8 samples could be prepared simultaneously. Standard samples were prepared using this mold.

All samples were prepared by manual mixing method. The specified amounts of antibiotic powders and 40 g of cement powder were homogenized by mixing thoroughly with a spatula. Then, cement liquid was added to this mixture and the cement was mixed for about 2 min. When the appropriate consistency of the prepared samples was achieved, they were poured into pre-prepared molds and allowed to solidify. The prepared samples were evaluated macroscopically and non-uniform faulty samples were excluded from the study. The samples were divided into six groups in total. Four 80×10×4 mm rectangular prism-shaped samples and one cylindrical sample with a height of 12 mm and a diameter of 6 mm for measuring antibiotic release were easily obtained from each 40-g cement batch. One of the groups was assigned as the control group and no antibiotics were applied to this group. Then, groups were formed by adding different doses of antibiotics to each 40 g of cement, as shown in Table I. Antibiotic concentrations were administered at doses similar to those reported in previous articles.[8,9]

Then, each group was separated according to antibiotic type and dose, and placed in separate containers containing phosphate-buffered saline (PBS) solution that mimics physiological

TABLE I
Antibiotic doses and percentages in 40 g cement

	Group 1		Group 2		Group 3		Group 4	
Type of antibiotic	g	%	g	%	g	%	g	%
Daptomycin	1	2.5	2	5	2.5	6.25	3	7.5
Vancomycin	0.5	0.125	2	5	4	10	6	15
Teicoplanin	0.8	2	1.2	3	2	5	2.4	6
Meropenem	0.5	0.125	2	5	4	10	6	15
Piperacillin-tazobactam	0.125	0.625	0.5	0.125	1	2.5	2	5

environments. To observe the effects of antibiotic release within each group under physiological conditions, samples were kept at 37°C for six weeks while PBS solutions were renewed every 24 h.

The mechanical tests were performed on specimens that were stored in PBS for 6 weeks, and the tests were carried out immediately after removal from PBS to mimic physiological conditions. During sample selection, specimens with visible macroscopic surface defects such as bubbles were excluded, and only those with smooth external surfaces were included in the study. At the end of six weeks, bending tests were applied to all samples with Instron® 3369 (Norwood MA, USA) brand test system under pressure up to 50 kN according to the groups they were assigned to. Measurements were made with a precision length gauge while applying the bending tests to the samples. The distance of the support points on the lower side was 60 mm, and the distance of the support points on the upper side was 20 mm (Figure 1). An electronic measuring device was used to avoid errors during locating the samples on the support points. The results of the bending tests were evaluated based on their force strength in MPa. The maximum force in MPa that they could withstand was measured based on the values at the point of failure.

The samples prepared for microbiological tests were placed in sterile containers and delivered to the Microbiology Laboratory. The samples reaching the laboratory were washed three times with physiological saline to remove cement dust residues. Then, the samples were transferred into sterile tubes and 5 mL of PBS solution was added. Totally 500-µL of sample was taken from the tubes at 1 h, 4 h, on Days 1, 3, 5, 7, 9, 11, 14, 18, and 21 and transferred in sterile Eppendorf tubes. The samples were stored at –20°C until the experiment day. After each sampling, 500-µL of PBS was added to the tubes. Before transfer into the microplates, PBS samples were filtered or centrifuged in order to eliminate potential interference from cement particles. The effectiveness of antibiotic-loaded cement samples was evaluated by liquid microdilution method. Standard 96-well U-bottom microplates were used for this assay. Mueller-Hinton Broth medium (90 µL) was added to each well. Samples were taken out from –20°C, thawed and 90 µL of each sample were



FIGURE 1. Four point bending test.

distributed to the corresponding wells containing the medium. After sufficient mixing was achieved, 90 µL of the sample-medium mixture was discarded, leaving 90 µL of sample-medium mixture in the wells. *Staphylococcus aureus* (ATCC 29213) strain was used for antibiotic-loaded cement samples containing vancomycin, teicoplanin and daptomycin, while *Pseudomonas aeruginosa* (ATCC 27853) strain was used for samples containing meropenem and piperacillin-tazobactam antibiotics. A 0.5 McFarland (1×10^8 CFU/mL) bacterial suspension was prepared from cultured *Staphylococcus aureus* and *Pseudomonas aeruginosa* passages. A new suspension containing $5x10^6$ CFU/mL bacteria was obtained by diluting the suspension at a 1:20 ratio. Bacterial suspension (10 µL) was added to each well and the final bacterial concentration in the wells was 5×10^5 CFU/mL. A well without bacterial suspension was used as a negative control, and a well without a sample was used as a positive control. At the end of the process, the microplates were placed in an incubator and incubated at 35°C for 16 to 20 h under aerobic conditions. At the end of this period, wells with visible turbidity were accepted as positive for bacterial growth. Specifically, macroscopic evaluation was performed at the end of incubation: in wells containing *Staphylococcus aureus* (ATCC 29213), a central pellet or button formation was considered positive for growth, while in wells containing *Pseudomonas aeruginosa* (ATCC 27853), either a pellet formation or diffuse turbidity in the absence of a pellet was accepted as evidence of bacterial growth.

A JEOL JSM-6060 LV (JEOL Inc., MA, USA) brand fully digital and computer-controlled

TABLE II
MPa values according to doses among groups

	n	Mean±SD	Median	Min-Max
Control (n=4)		54.29±2.74	54.51	50.92-57.23
Daptomycin (g)				
1	4	51.07±4.17	51.04	46.13-56.08
2	4	49.54±3.39	49.84	45.22-53.27
2.5	4	41.61±5.18	42.28	34.81-47.06
3	4	31.21±3.06	30.78	28.15-35.12
Vancomycin (g)				
0.5	4	50.40±3.16	51.04	46.13-56.08
2	4	45.02±5.98	44.35	39.54-51.83
4	4	33.33±2.72	33.16	30.18-36.81
6	4	24.61±5.67	24.95	18.13-30.41
Meropenem (g)				
0.5	4	52.08±2.08	52.48	49.24-54.13
2	4	49.81±2.55	49.70	46.84-53.01
4	4	39.73±6.42	39.61	32.91-46.79
6	4	31.18±3.02	30.82	27.88-35.20
Piperacillin-tazobactam (g)				
0.25	4	51.29±0.93	51.39	50.13-52.24
0.5	4	50.21±1.94	50.92	47.34-51.65
1	4	44.53±3.13	44.53	41.53-47.51
2	4	38.56±5.05	38.05	33.91-44.23
Teicoplanin (g)				
0.8	4	50.69±1.14	50.64	49.35-52.13
1.2	4	43.13±3.89	43.32	38.19-47.69
2	4	40.09±6.28	41.03	31.83-46.48
2.4	4	36.52±6.41	38.46	27.39-41.76

SD: Standard deviation.

TABLE III
Dose-MPa measurement relationship in AB groups

	n	r	*p*
Daptomycin	16	−0.883	<0.001
Vancomycin	16	−0.920	<0.001
Meropenem	16	−0.914	<0.001
Piperacillin-tazobactam	16	−0.889	<0.001
Teicoplanin	16	−0.889	<0.001

MPa: Megapascals. If Spearman correlation coefficient (the absolute value of the correlation coefficient [rho]) is r≤0.30, there is a weak relationship; if it is between 0.30-0.50, there is a moderate relationship; and if r≥0.50, there is a strong relationship.[1]

electron microscope was used to compare the control sample and randomly selected 5% portions of antibiotic-loaded cements with electron microscopy.

Statistical analysis

Statistical analysis was performed using the SPSS for Windows version 22.0 software (IBM Corp., Armonk, NY, USA). Conformity of continuous variables to normal distribution was assessed using histogram and probability graphs and Kolmogorov-Smirnov and Shapiro-Wilk tests. Results of the normality analysis determined that the continuous variables were not normally distributed. Continuous variables were presented in mean ± standard deviation (SD) or median (min-max). The significance of the differences measured between pairwise comparisons of the groups in terms of continuous variables was analyzed using the two-tailed Mann-Whitney U test. The relationship between antibiotic dose and MPa measurements was evaluated with the Spearman correlation test. A p value of <0.05 was considered statistically significant.

RESULTS

The results of the bending tests on different antibiotic doses examined in our study showed that MPa measurements decreased as the antibiotic dose in the mixture increased, regardless of the antibiotic type (Table II). The relationship between the antibiotic dose in the mixture and MPa was examined in the antibiotic groups, and a statistically significant strong negative correlation was found between the dose and MPa for all antibiotic types (Table III). According to these results, MPa values decreased significantly in all antibiotic groups proportionally with increasing antibiotic dose. When the mechanical strength was examined with respect to the antibiotic dose, mixing antibiotics at more than 5% concentration significantly reduced mechanical strength. For piperacillin-tazobactam, 2.5% antibiotic-loaded bone cement remained below the standard value of 50 MPa, while for other antibiotics, mixing antibiotics into cement at 2.5% concentrations did not cause significant changes in mechanical measurements.

To evaluate the relationship between antibiotic type and mechanical strength, MPa values were recorded for 2-g doses of each antibiotic type. Comparative analysis revealed that MPa values in the daptomycin 2 g group were statistically significantly higher than the piperacillin-tazobactam and teicoplanin 2 g groups (p=0.021 and p=0.043, respectively). Similarly, MPa values in the meropenem 2 g group were found to be statistically significantly higher

TABLE IV
Evaluation of MPa levels at 2 gram doses among groups

	Mean±SD	Median	Min-Max	*p*[1]
Daptomycin (G1)	49.54±3.39	49.84	45.22-53.27	G1 *vs.* G2: 0.248
				G1 *vs.* G3: 1.000
Vancomycin (G2)	45.02±5.98	44.35	39.54-51.83	G1 *vs.* G4: **0.021**
Meropenem (G3)	49.81±2.55	49.70	46.84-53.01	G1 *vs.* G5: **0.043**
Piperacillin-tazobactam (G4)	38.56±5.05	38.05	33.91-44.23	G2 *vs.* G3: 0.248
				G2 *vs.* G4: 0.248
				G2 *vs.* G5: 0.248
Teicoplanin (G5)	40.09±6.28	41.03	31.83-46.48	G3 *vs.* G4: **0.021**
				G3 *vs.* G5: **0.021**
				G4 *vs.* G5:0.773

SD: Standard deviation; [1]Mann-Whitney U test.

than the piperacillin-tazobactam and teicoplanin 2 g groups (p=0.021 and p=0.021, respectively). There were no statistically significant differences between the applications of 2 g doses of antibiotics (Table IV).

Samples from all groups were kept in PBS solution for six weeks; afterward, 5% of antibiotic-loaded cement samples were randomly selected. Selected 5% samples were, then, examined under an electron microscope at 100× and 250× magnifications. In the analysis of these images, it was noteworthy that the gaps, particularly in the piperacillin-tazobactam group and the teicoplanin group were much larger than those in the meropenem, daptomycin, and control groups. The fact that these gaps were observed more frequently in these two antibiotic-loaded cement groups supports our finding that these groups were mechanically weaker than the other three antibiotic mixtures.

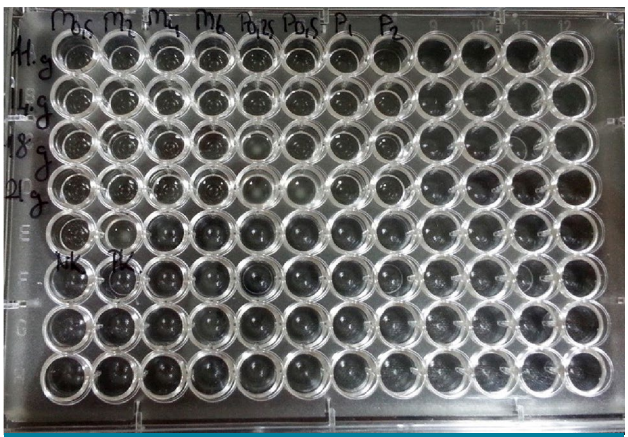


FIGURE 2. Control of bacterial growth between 11th and 21st days on U-bottom standard microplates.

NK: Negative control (a well without bacterial suspension), PK: Positive control (a well without sample); M0.5: Meropenem 0.5 g; M2: Meropenem 2 g; M4: Meropenem 4 g; M6: Meropenem 6 g; P0.25: Piperacillin-Tazobactam 0.25 g; P0.5: Piperacillin-Tazobactam 0.5 g; P1: Piperacillin-Tazobactam 1 g; P2: Piperacillin-Tazobactam 2 g; 11 g: 11th day; 14 g: 14th day; 18 g: 18th day; 21 g: 21st day. There was growth in the sample of the Piperacillin-Tazobactam group containing 0.25 gr, on 18th and 21st days; in the sample of the Piperacillin-Tazobactam group containing 0.5 g, on the 21st day; and in positive control group, while no growth was observed in other groups.

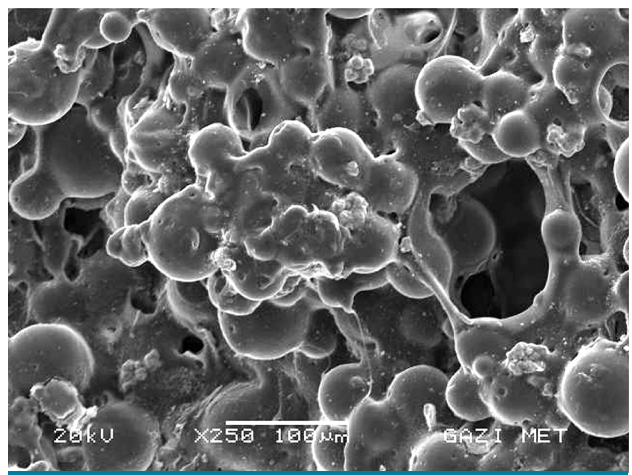


FIGURE 4. Scanning electron microscopy image of bone cement loaded with 5% teicoplanin at 250× magnification. Note the increased porosity and void formation compared to the control sample, which correlates with reduced mechanical strength.

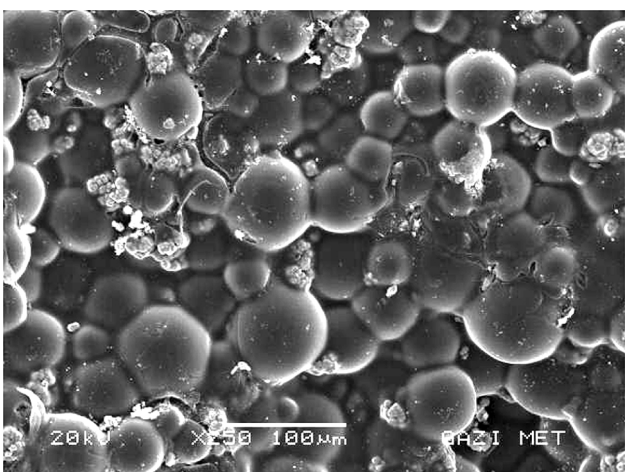


FIGURE 3. Scanning electron microscopy image of control bone cement at 250× magnification. The image shows the typical microstructure of plain polymethylmethacrylate bone cement with relatively uniform polymer beads and minimal porosity.



FIGURE 5. Scanning electron microscopy image of bone cement loaded with 5% piperacillin-tazobactam at 250× magnification. The image demonstrates extensive pore formation and microstructural disruption, consistent with the observed mechanical weakness in this antibiotic group.

Scanning electron microscopy (SEM) analysis revealed distinct microstructural differences between the control and antibiotic-loaded cement samples (Figures 3-5). The control cement displayed a relatively uniform structure with minimal porosity (Figure 3). In contrast, antibiotic-loaded samples showed increased void formation, with teicoplanin (Figure 4) and piperacillin-tazobactam (Figure 5), indicating the most pronounced structural disruption. These microstructural changes provide a mechanistic explanation for the observed reductions in mechanical strength.

No bacterial growth was observed at the end of Day 21 in the wells of antibiotic-loaded cement samples containing vancomycin at concentrations of 0.5, 2, 4, and 6 g; teicoplanin at concentrations of 0.8, 1.2, 2, and 2.4 g; daptomycin at concentrations of 1, 2, 2.5, and 3 g; and meropenem at concentrations of 0.5, 2, 4, and 6 g. While growth was observed on Days 18 and 21 in the wells of antibiotic-loaded cement samples containing piperacillin-tazobactam at a concentration of 0.25 g, growth was observed only on Day 21 for the same antibiotic samples at 0.5-g concentration. No growth was observed at the end of Day 21 in the wells of antibiotic-loaded cement samples containing piperacillin-tazobactam at concentrations of 1 and 2 g (Figure 2).

DISCUSSION

In the current study, we determined the antimicrobial efficacy and mechanical strength of antibiotic-loaded cement samples in which daptomycin, vancomycin, teicoplanin, meropenem and piperacillin-tazobactam agents were mixed at different doses. Our study results showed that cements with 5% concentrations of daptomycin and meropenem exhibited sufficient efficacy both mechanically and microbiologically. Cements with vancomycin and teicoplanin lost their mechanical strength faster at increasing doses, while piperacillin-tazobactam lost its mechanical strength when it reached microbiologically effective doses. To the best of our knowledge, the present study is one of the first studies showing the microbiological and mechanical effects of piperacillin-tazobactam when added to bone cement.

In previous studies, factors affecting antibiotic release from different bone cements, such as antibiotic type and amount, cement porosity, environmental conditions, and cement preparation, have been extensively studied.[10,11] However, no publication has yet clearly shown the maximum antibiotic concentration that can be loaded without affecting the mechanical properties of cements. However, many studies have shown that mechanical properties were affected to an acceptable extent when the added antibiotic concentration was low (between 5% and 10%).[12,13] In addition, studies have reported that as the doses of antibiotics added to cements increased, mechanical strength deteriorated.[11-13] However, most of these studies were carried out without considering the effect of physiological fluids on cement.

In the current study, the physiological effects to which the cement would be exposed during the six weeks between the stages of two-stage revision arthroplasty surgery were also investigated in terms of the mechanical and microbiological effectiveness of antibiotic cement. We observed that the flexural mechanical strength of cements with antibiotic contents of more than 2.5% decreased below the minimal value of 50 MPa, particularly when antibiotic-loaded cements were kept at body temperature for six weeks in PBS solution. Taken together, these results demonstrate that the mechanical properties of cement are highly dependent on the loaded antibiotic concentration.

Nelson et al.[14] and Pelletier et al.[8] found significant differences in the mechanical properties and cement behavior between cements that were and were not kept in PBS solution. In general, no significant changes were detected in the mechanical properties of antibiotic-loaded cements that were not kept in PBS solution after antibiotic addition, regardless of antibiotic concentration and type used. However, after cements were kept in PBS solution, significant decreases in strength were observed in all cement groups. These strength reductions varied depending on the dose and type of antibiotic. Similarly, the present study showed that cements kept in PBS lost their mechanical strength at concentrations below the 5-10% level, contrary to results from isolated mechanical tests.

Loading different antibiotics into cements at different percentages results in different mechanical strengths. Studies have reported that vancomycin creates a greater reduction in mechanical strength than meropenem, particularly when added to bone cement at concentrations exceeding 2.5%.[15] Different types of antibiotics incorporated into bone cement exhibit variations not only in their antibiotic release profiles but also in their capacity for liquid absorption. In one

study, different fluid uptakes were observed in cements mixed with the same amounts of cefazolin and vancomycin, which can be attributed to differences in molecular weights of the antibiotics and particle morphology.[9]

Vancomycin, teicoplanin, and daptomycin, antibiotics effective against Gram-positive bacteria, were found to have sufficient antibacterial activity; however, at a 5% concentration, the mechanical strength of vancomycin-loaded cement decreased to significantly lower MPa values. Some studies have reported that there was no antibiotic release after Day 14 with daptomycin, particularly at low doses.[16] However, in our study, daptomycin maintained its antibacterial properties for 21 days.

Currently, multidrug-resistant Gram-negative infections are frequently encountered in clinical practice. These infections usually have an acute onset, and Gram-negative infections are usually considered more difficult to treat than Gram-positive infections. Gram-negative organisms account for 10 to 20% of implant-associated orthopedic infections. In our study, we observed that piperacillin-tazobactam, despite its activity against Gram-negative bacteria, showed insufficient antibacterial properties, particularly at low doses (0.25 g and 0.5 g). However, piperacillin-tazobactam used at concentrations above 0.5 g might show adequate antibacterial activity. However, mechanical measurements revealed that 1-g piperacillin-tazobactam-loaded cement was also mechanically inadequate. Therefore, using piperacillin-tazobactam alone in antibiotic-loaded cements is not considered appropriate. To the best of our knowledge, no study in the literature has comprehensively examined the antibacterial activity and mechanical strength of piperacillin-tazobactam yet.

In the current study, meropenem, an antibiotic effective against Gram-negative bacteria, showed effective antibacterial and mechanical properties at concentrations of 5% and below. This 21-day period during which meropenem maintained its antibacterial effect at different doses, has also been demonstrated in previous studies, consistent with our findings.[17] When choosing between these two antibiotics according to the resistance status of Gram-negative bacteria, meropenem may be preferred over piperacillin-tazobactam due to its superior antibacterial properties and greater mechanical strength. These changes in mechanical properties were probably directly related to the liquid absorption process. Greater fluid absorption and antibiotic release could lead to increased pore formation and microcracking.

Periprosthetic joint infection is a serious complication following total hip arthroplasty. A case report, although the current literature offers limited support for alternatives to the conventional two-stage revision in Salmonella prosthetic joint infections, demonstrates that the custom-made articulating spacer technique can be a safe and effective treatment strategy when combined with targeted intraoperative antibiotic-loaded cement and appropriate postoperative antibiotic therapy.[18]

Nonetheless, the present study has some limitations. First, as in other studies,[9,19,20] we attempted to simulate body conditions by keeping the cements in PBS solution. However, different substances such as proteins and glycoproteins are present in the synovial fluid of a healthy joints, and these may affect the cement properties. Therefore, these factors should be considered while applying study findings to clinical practice. Second, in our study, antibiotics were added to cements individually, and the synergistic effects of different antibiotics and their potential interactions were not evaluated, unlike some recent studies.[9,20] Moreover, the antimicrobial effect was evaluated qualitatively based on the presence or absence of turbidity, without applying quantitative methods such as high-performance liquid chromatography or inhibition zone measurements, which limits characterization of release kinetics. Also, the mechanical tests were conducted with a relatively small sample size (n=4 per group), mainly due to laboratory and resource constraints, and no formal power analysis was performed; therefore, the mechanical results should be interpreted with caution. Finally, force-displacement curves were not recorded during bending tests and SEM images were assessed qualitatively without quantitative porosity analysis, restricting the ability to fully correlate microstructural changes with mechanical strength.

Future studies should include larger sample sizes to improve the reproducibility of mechanical test results and confirm our findings. Since our results demonstrated that increasing antibiotic doses, particularly above 5%, significantly reduced mechanical strength, further research is needed to determine the optimal antibiotic concentrations that balance antimicrobial

efficacy with mechanical safety. In addition, while our study investigated single antibiotics individually, the synergistic or antagonistic effects of antibiotic combinations should be evaluated in future experiments. Quantitative techniques such as high-performance liquid chromatography may provide a more detailed understanding of antibiotic release kinetics. Finally, *in vivo* studies are required to validate the clinical applicability of these results.

In conclusion, different types of antibiotics may cause varying degrees of mechanical strength loss during incubation in PBS. Considering the antimicrobial activities of antibiotics at different doses that we have applied, it is of utmost importance to select appropriate doses of antibiotics to protect the mechanical strength of the cement, to avoid cost, and prevent toxic effects resulting from high antibiotic concentration. In particular, the need to find a balance between the mechanical properties of the cement and the expected therapeutic effect of the antibiotic is imperative.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conceptualization, T.E., M.A.T., H.Y.S.; Methodology, visualization: T.E., T.C.; Validation, T.E., H.Y.S., M.A.T.; Formal analysis, T.C., A.E.Y.; Investigation, supervision, writing, review and editing: T.E., H.Y.S.; Resources, writing, original draft preparation: T.E.; Data curation, T.E., A.E.Y.; Funding acquisition, H.Y.S. All authors have read and agreed to the published version of the manuscript.

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