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Is Gentamicin Elution Influenced by the Timing of Antibiotic Addition to the Bone Cement? An In Vitro Study on Articulating Hip Spacers

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Abstract: Periprosthetic Joint Infection (PJI) is a challenging complication after joint replacements, and cement spacers are standard treatment in two-stage revision surgery. This experimental in vitro study aimed to evaluate the elution properties of different gentamicin formulations from commercially available intraoperative molded hip cement spacers. The study compared spacers prepared with premixed antibiotic bone cement, spacers with antibiotic powder added during preparation, spacers stored for two months, and spacers with an additional antibiotic. The results showed that the timing of antibiotic addition influenced gentamicin elution, with immediate elution resulting in higher levels than stored spacers. Spacers with antibiotic powder added during preparation exhibited higher elution than premixed antibiotic spacers. Furthermore, adding vancomycin to the bone cement significantly increased gentamicin elution. These findings suggest that optimizing the timing and method of antibiotic addition in cement spacers may enhance the effectiveness of antibiotic treatment in PJI. However, further research is needed to validate these findings and explore their clinical implications.

Keywords: antibiotic-loaded bone cement; hip spacer; gentamycin elution; antibiotic kinetics

Citation: Cacciola, G.; Bosco, F.; Giustra, F.; De Meo, F.; Bruschetta, A.; Sabatini, L.; Artiaco, S.; Giraldo, D.; Massè, A.; Cavaliere, P. Is Gentamicin Elution Influenced by the Timing of Antibiotic Addition to the Bone Cement? An In Vitro Study on Articulating Hip Spacers. *Prosthesis* **2023**, *5*, 952–961. https://doi.org/10.3390/prosthesis5030066

Academic Editor: Marco Cicciu

Received: 21 July 2023
Revised: 8 September 2023
Accepted: 11 September 2023
Published: 12 September 2023

1. Introduction

Periprosthetic Joint Infection (PJI) is one of the most challenging complications after joint replacements. Its incidence is about 1% after primary hip replacement and 2.5% after primary knee replacement [1]. Its high perioperative costs, perioperative mortality, and poor outcomes after reimplantation make it one of the most feared complications [2]. Nowadays, the management of periprosthetic joint infections is equally focused on the eradication of the infection and the preservation of a functional joint throughout the treatment period. An early postoperative infection or an acute hematogenous infection may be amenable to a debridement and implant retention procedure, while a two-stage arthroplasty exchange would be preferable for a late chronic infection [2,3]. Once the infected implant is removed, debridement and substitution of necrotic and granulation tissue with antibiotic-impregnated cement articulating or static spacers is a standard interim therapy to improve the outcome of re-implantation and prevent recurrence of the infection. Antibiotic-loaded cement spacers could offer a higher success rate of infection control due to increased local concentrations of antibacterial agents with minimal effects on serum or urine antibiotic levels [2,3]. Apart from that, these spacers maintain joint space and stability.

Local delivery provides substantially high concentrations of the antibiotic at the site of poorly vascularized infected bone, which is inaccessible to even high doses of systemic intravenous antibiotics [3,4].

Despite the increasing use of one-stage revision over the past year, two-stage revision using a temporary, antibiotic-infused cement spacer is still considered the gold-standard treatment for almost all cases of PJI [3,4]. Initially, spacers were static (or blocks of cement) with the sole objective of locally delivering high concentrations of antibiotics. In recent years, there has been a shift towards articulated spacers over static spacers [5,6]. Using articulated spacers could also provide mechanical support, allowing partial weight-bearing, maintaining range of motion (ROM), and ensuring adequate soft tissue preservation between the first and second stages [7]. The clinical effectiveness of two-stage revision in eradicating infections has been extensively studied, with several prospective studies and systematic reviews highlighting its efficacy [8,9]. However, only a few studies have evaluated the elution of antibiotics from cement spacers in vitro and in vivo [10–12].

Another way to classify cement spacers is based on their method of creation. These include hand-made spacers created by the surgeon during surgery, prefabricated spacers prepared and stored before being implanted, and intraoperatively molded spacers prepared during surgery using a mold [13]. Intraoperatively molded hip spacers provide numerous advantages in managing PJI during two-stage revision surgery. The customization of spacer size and shape improves joint stability [14]. These spacers enable the incorporation of appropriate antibiotics into the cement based on the results of antibiotic susceptibility tests. This tailored approach ensures effective infection control and the reduction of mechanical complications and contributes to better patient outcomes [15].

The primary objective of the present study is to evaluate whether the timing of spacer preparation influences the elution of gentamicin from cement spacers. We hypothesize that "stored" spacers will have diminished antibiotic elution capacity compared to spacers prepared during surgery. As a secondary objective, we aim to assess whether spacers designed with premixed bone cement containing antibiotics exhibit different pharmacokinetics than those prepared with plain bone cement and powdered gentamicin. Additionally, we seek to determine if the synergistic effect observed with adding two antibiotics to bone cement is also evident in spacers.

2. Materials and Methods

2.1. Study Design

This experimental in vitro study compared the elution properties of different gentamicin formulations from commercially available intraoperative molded hip cement spacers (Spaceflex Hip by G21, San Possidonio, Modena, Italy). Spacers were prepared with Spaceflex Hip instrumentation using G3 and G3A low-viscosity antibiotic bone cement (G21 srl, San Possidonio, Modena, Italy). G3A is a low-viscosity bone cement (40 g of PMMA per pack) premixed with 1 g of gentamicin (gentamicin sulfate), while G3 (40 g of PMMA per pack) is a low-viscosity bone cement without the addition of antibiotics. Each spacer was prepared with three packs of G3A or G3 types of cement. The Spaceflex hip is an articulated modular spacer with the possibility to choose between three different sizes of the femoral head and three different measures of stem length and stem diameter. All the spacers were prepared with the same sizes: femoral head 48 mm, femoral length 170 mm, and femoral width 13 mm. Vacuum polymerization was not used during the preparation of the cement spacer. (2/1 polymer powder/liquid monomer ratio) as suggested by the producer [5].

We created four different experimental models, with five spacers prepared for each model (Table 1). The first spacers (Spacer #1), premixed cement/antibiotic spacers, were designed using three packs of G3A bone cement. The second group (Spacer #2) consists of pale cement with the addition of antibiotic powder. These spacers were created using three packs of G3 bone cement and adding 3 g of gentamicin (gentamicin sulfate) powder during spacer preparation. The third group (Spacer #3), preformed spacers, were prepared similarly to Spacer #1 but underwent sterilization with ethylene oxide and were stored

for two months before evaluating the elution of gentamicin. The spacers were kept in a refrigerator at 3 °C until their analysis in a closed container. The fourth group (Spacer #4), known as double antibiotic spacers, was prepared like Spacer #1 but with the addition of 3 g of vancomycin powder. The polymerization reaction of PMMA was performed without a vacuum to increase the number and size of pores within the bone cement, facilitating the elution of antibiotics. All spacers were made in a sterile environment with a room temperature of 23 ± 1 °C.

Table 1. Description of the composition of the different spacers. For each group, five different spacers were prepared.

Spacer Type	Bone Cement	Antibiotic	Timing of Addition of the Antibiotic
Spacer #1	3 packs of G3A	3 g Gentamycin	Premixed with the bone cement
Spacer #2	3 packs of G3	3 g Gentamycin	Antibiotic powder mixed with bone cement at spacer preparation
Spacer #3	3 packs of G3A	3 g Gentamycin	Premixed with bone cement, stored two months before elution evaluation
Spacer #4	3 packs of G3A	3 g Gentamycin and 3 g vancomycin	Gentamycin was premixed with the bone cement, while vancomycin was added at spacer preparation

2.2. Description of the Experimental Model

The liquid medium was prepared by dissolving one pouch of phosphate-buffered saline (PBS) in a 1-L flask filled with demineralized water, resulting in a pH 7.4 buffered saline solution. The solution was then transferred to a tank and stored in the refrigerator. Next, the liquid medium (PBS) was placed in an incubator and heated to 37 ± 1 °C.

Once the liquid medium reached the desired temperature, four beakers were filled with 1 L of the solution (the elution volume). The spacers were placed in separate beakers, and efforts were made to remove any surface bubbles by gently moving and shaking the beakers. The test was conducted in an incubator, whose temperature was set to obtain an extraction temperature of 37 ± 1 °C. The starting time was then recorded on each beaker. The antibiotic diffusion data were analyzed at the following time markers: T0 = 0 h, T1 = 24 h, T2 = 1 w, and T3 = 1 m.

At each time, three samples of the medium solution were collected using a pipette, each consisting of 250 µL. These samples were then transferred to separate Falcon tubes. To facilitate the circulation of the liquid medium around the specimen, manual agitation of the liquid medium was initiated 30 s before each time marker. Again, at each time, three samples of the medium solution were collected using a pipette and placed in separate falcon tubes. Liquid chromatography-tandem mass spectrometry equipment (LC-MS/MS) was used to measure the concentration of the eluted antibiotic in the liquid medium [16].

2.3. Objectives of the Study

The primary objective of this study is to investigate the potential influence of the timing of antibiotic addition in bone cement on gentamicin elution. We compared antibiotic elution in different experimental models to examine this hypothesis. Firstly, we reached Spacers #1 and #2, prepared with G3A bone cement (premixed with 1 g of gentamicin powder) and G3 bone cement with 1 g of gentamicin powder during spacer preparation, respectively. We compared Spacers #1 and #3, prepared with the same bone cement (G3A). Still, the elution from Spacer #1 was recorded immediately, while the elution from Spacer #3 was assessed after storing them for two months. Lastly, we compared Spacers #1 and #4, prepared with the same bone cement (G3A), but Spacer #4 had an additional antibiotic (1 g of vancomycin per pack of bone cement).

2.4. Statistical Analysis

The data was collected and recorded in an Excel worksheet (Version 16.16.27 by Microsoft, Redmond, WA, USA) and subsequently imported into Prism version 7.0

for MacOS (by GraphPad, San Diego, CA, USA) for statistical analysis. The elution of gentamicin at each time interval was assessed using an independent t-test to compare the gentamycin elution in different conditions. We used an analysis of variance (ANOVA) to determine the statistical difference in gentamycin elution from spacers with different preparation methods.

3. Results

3.1. Cumulative Gentamicin Elution

The overall cumulative elution of gentamicin from Spaceflex hips is listed in Table 2. Spacer #4 showed the highest cumulative gentamicin elution, with an average value of 20.3 mg/L SD 5.1 at one month. The total cumulative gentamicin elution was statistically significantly higher for Spacer #4 compared to the other three groups at each time marker ($p < 0.01$). It was considering the other three groups of spacers containing gentamicin only. The highest elution was observed for Spacer #2, with a peak of gentamicin elution of 8.6 mg/L, SD 2.1, at one month. The worst elution was reported for Spacer #1, with its highest value of 5.7 mg/L SD 1.7 at one month.

Table 2. Cumulative elution of gentamycin from different models of hip spacer.

		Spacer #1		Spacer #2		Spacer #3		Spacer #4	
Intervals of Time		Gentamycin Elution Detected (mg/L)	SD	Gentamycin Elution Detected (mg/L)	SD	Gentamycin Elution Detected (mg/L)	SD	Gentamycin Elution Detected (mg/L)	SD
T0	0 h	0	0	0	0	0	0	0	0
T1	24 h	3.5	1.1	6.8	2.1	3.0	1.0	15.5	4.2
T2	1 w	5.3	1.5	7.6	1.7	3.8	1.3	18.4	4.7
T3	1 m	5.7	1.7	8.6	2.1	5.9	1.6	20.3	5.1

Abbreviations: SD: standard deviation; mg: milligram; l: liters; T: time; h: hour; w: week; m: month.

3.2. Comparison between Spacers #1 and #2

We compared the gentamicin elution between the two spacers prepared with the same bone cement, one premixed with gentamicin (Spacer #1) and the other one without premixed gentamicin (Spacer #2). The gentamicin powder was added during the spacer preparation in the second group (Figure 1). A significant increase in gentamicin elution was reported for Spacer #2 at each time marker. At 24 h, the average cumulative gentamicin elution was 3.5 mg/L SD 1.1 for Spacer #1 and 6.8 mg/L SD 2.1 for Spacer #2 ($p = 0.137$). At one week, the average cumulative gentamicin elution was 5.3 mg/L SD 1.5 and 7.6 mg/L SD 1.7 ($p = 0.049$), respectively. At one month, it was 5.7 mg/L SD 1.7 and 8.6 mg/L SD 2.1, respectively ($p = 0.042$).

3.3. Comparison between Spacers #1 and #3

We compared the gentamicin elution between two spacers prepared with the same bone cement (G3A bone cement). In Spacer #1, the gentamicin elution was collected immediately, while in Spacer #3, it was collected after a storage period of two months (Figure 2). Spacer #1 demonstrated increased elution concentration at one week compared to Spacer #3 (5.3 mg/L SD 1.5 and 3.8 mg/L SD 1.3, respectively, $p = 0.032$). Spacer #1 showed increased elution concentrations after 24 h, but it was not statistically significant (respectively, 3.5 SD 1.1 and 3.0 mg/L SD 1.0, $p = 0.084$). In contrast, Spacer #3 presented more gentamicin elution than Spacer #1 after one month, but it was not statistically significant (5.7 mg/L SD 1.7 for Spacer #1 and 5.9 mg/L SD 1.6 for Spacer #3; $p = 0.134$).

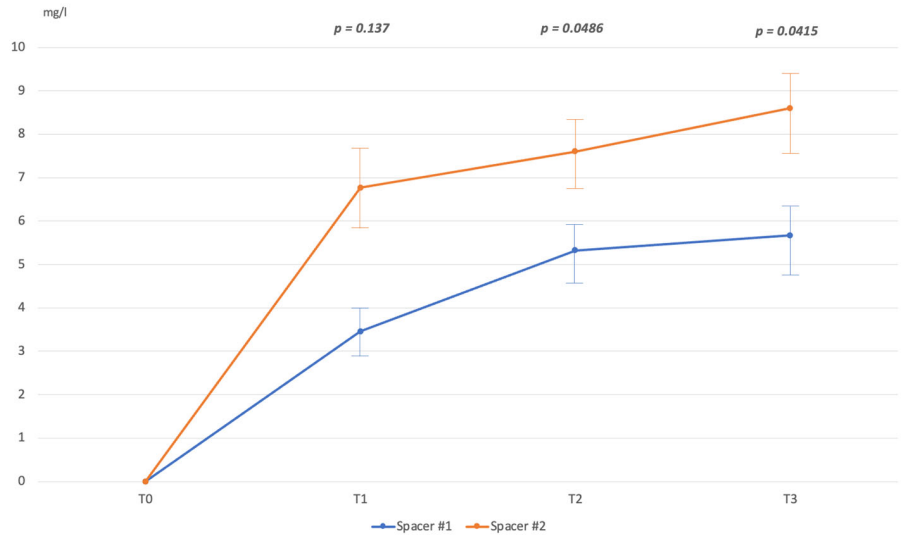

Figure 1. Comparison of cumulative gentamycin elution between Spacers #1 and #2. The spacer of the second group showed a statistically significant increase in gentamycin elution at each interval of time. Abbreviations: SD: standard deviation; mg: milligram; l: liters; *p*: *p*-value.

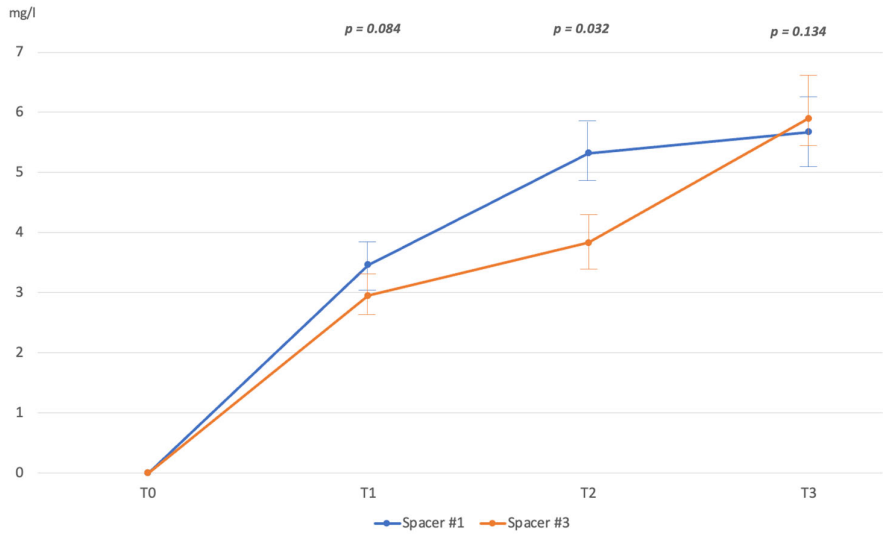

Figure 2. Comparison of cumulative gentamycin elution between Spacers #1 and #3. The spacer of the third group showed a statistically significant increase in gentamycin elution at 1 week only. Abbreviations: SD: standard deviation; mg: milligram; l: liters; *p*: *p*-value.

3.4. Comparison between Spacers #1 and #4

We compared the gentamicin elution between two spacers prepared with the same bone cement (G3A bone cement), with the only difference being that in Spacer #4, 1 g of vancomycin was added for each pack of bone cement (Figure 3). Spacer #4 showed a statistically significant increase in cumulative gentamicin elution at each time marker. At 24 h, Spacer #4 showed an increased elution of gentamicin of 447.8% compared to Spacer #1 (3.5 mg/L SD 1.1 for Spacer #1 and 15.5 mg/L SD 4.2 for Spacer #4, $p < 0.001$). At one week, Spacer #4 showed an average increased elution of gentamicin of 345.8% compared to Spacer #1 (18.4 mg/L SD 4.7, 5.3 mg/L SD 1.5, and $p < 0.001$, respectively). Similar results were also recorded for one month, in which Spacer #4 showed an average increased elution of gentamicin of 358% compared to Spacer #1 (20.3 mg/L SD 5.1 and 5.7 mg/L SD 1.7, $p < 0.001$; respectively).

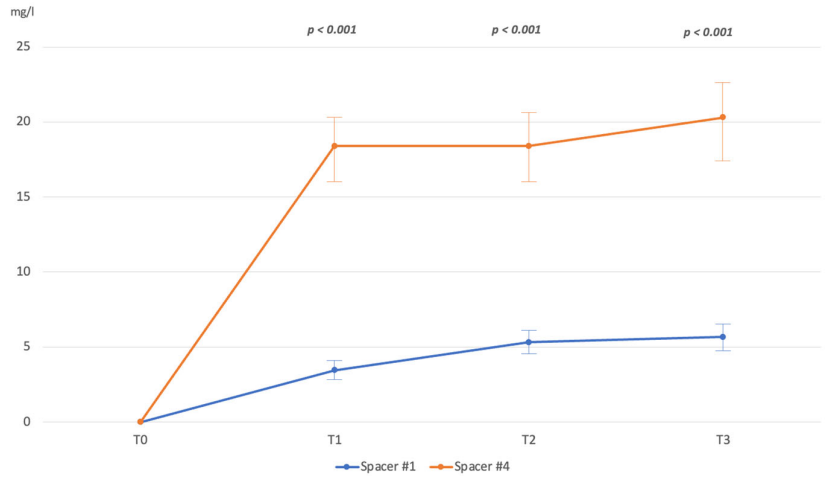

Figure 3. Comparison of cumulative gentamycin elution between Spacers #1 and #4. The spacer of the fourth group showed a statistically significant increase in gentamycin elution at each interval of time. Abbreviations: SD: standard deviation; mg: milligram; l: liters; *p*: *p*-value.

3.5. Comparison of Gentamycin Elution between Groups

Based on the ANOVA test, there is evidence to suggest that there are statistically significant differences among the average cumulative gentamicin elution at 24 h ($p = 0.036$), at 1 week ($p = 0.006$), but not after one month ($p = 0.105$) for the different groups.

4. Discussion

This study's most important finding is that adding vancomycin to the bone cement (Spacer #4) resulted in a significantly higher cumulative gentamicin elution than the other groups. Spacer #4 showed the highest cumulative gentamicin elution at one month, with an average value of 20.3 mg/L. This finding suggests that adding vancomycin to the bone cement may enhance the elution of gentamicin and can potentially improve the effectiveness of antibiotic treatment in PJI. In addition, this study demonstrated that the moment of the addition of gentamicin to the spacer preparation influences its elution. Spacers prepared with bone cement with gentamicin mixed at the time of spacer preparation (Spacer #2) exhibited higher elution than those with gentamicin powder premixed with bone cement (Spacer #1). Furthermore, with "freshly made" spacers, immediate collection of gentamicin elution after one week (Spacer #1) resulted in significantly higher elution after one week compared to the premade spacers stored for two months before the sample collection (Spacer #3). These findings suggest that the moment of spacer preparation and storage duration may affect the elution characteristics of antibiotics from cement spacers.

The results indicate that adding the antibiotic to the cement during the spacer preparation (Spacer #2) significantly increased gentamicin elution compared to spacers with premixed gentamicin (Spacer #1). Spacer #2 consistently showed higher gentamicin elution levels at each time marker. These results suggest that adding gentamicin powder during spacer preparation, rather than using premixed gentamicin-containing bone cement, leads to higher gentamicin elution levels [17–19]. This finding highlights the importance of the timing and method of antibiotic addition in influencing the release of antibiotics from bone cement. It may have implications for optimizing antibiotic therapy in spacer-related treatments. Regardless of this study's findings, it is essential to acknowledge delivery vehicles, types of antibiotics, dosage recommendations, indications, and tribological and mechanical properties when preparing antibiotic-loaded spacers or bone cement [20,21]. There is a difference in elution between powdered antibiotics and liquid antibiotics [22,23].

Liawrungrueand et al. [22] conducted a similar study comparing the elution of gentamicin from cement spacers using powdered gentamicin versus liquid gentamicin. Their findings indicated that liquid gentamicin exhibited a twofold higher elution than powdered gentamicin. However, it is essential to note that several authors advise against incorpo-

rating liquid antibiotics into bone cement because it will significantly alter the cement's mechanical properties [24–26]. Therefore, a thorough assessment of the balance between elution efficiency and cement integrity is necessary when combining antibiotics with bone cement [24–26]. Other important factors influencing antibiotic elution from bone cement are the number and size of pores within the cement matrix [27]. When the antibiotic is incorporated into the cement, it can occupy larger pores, increasing the available surface area for its release.

Consequently, antibiotics trapped within larger pores can be more easily released into the surrounding tissue, resulting in higher elution rates [27]. A higher number of pores in the bone cement creates additional pathways for antibiotic diffusion and release. Increased pore density allows for a greater distribution of antibiotics throughout the cement matrix [28]. Therefore, more opportunities exist for the antibiotic to be released from multiple locations, leading to higher elution rates. Combining both factors—more significant and increased pore density within the bone cement—creates an interconnected network that will enhance the diffusion and elution of antibiotics, ultimately increasing the overall elution rate. For this reason, we suggest preparing the spacers without a vacuum during the polymerization reaction [23,28].

Another important finding of this study is that the gentamicin elution from two different spacers prepared with the same bone cement (G3A) is influenced by the "time of the evaluation". We demonstrated that Spacer #1 (prepared and immediately tested for gentamicin elution) showed a higher gentamicin elution at one week compared to Spacer #3 (prepared in the same way but stored for two months before evaluation). There was also an increased but not statistically significant gentamicin elution after 24 h. However, at one month, the gentamicin elution was slightly lower for Spacer #1 compared to Spacer #3, but it was not statistically significant. Several possible explanations exist for the reduction in gentamicin elution due to storage [29,30]. First, antibiotic molecules may gradually diffuse into the surrounding environment, reducing the concentration and availability of gentamicin for elution during its use. Second, gentamicin molecules could interact with other components in the bone cement, leading to decreased availability for elution. Third, antibiotics may undergo degradation and chemical changes over time. Lastly, as bone cement undergoes a process of hydration over time, the antibiotic within it may become less available for elution. All these factors contribute to a decrease in the availability of gentamicin when a spacer is stored [31–33].

In general, antibiotic-loaded bone cement or cement spacers are prepared with a single antibiotic, often an aminoglycoside such as gentamicin or tobramycin. These antibiotics are effective against Gram-negative bacteria present in PJI. However, they may not be fully effective against Gram-positive bacteria, which account for more than 80% of PJI cases [34,35]. Many authors suggest adding a second antibiotic for two reasons. The first reason is to widen the spectrum of activity of the antibiotic therapy, while the second reason is that adding a second antibiotic increases the elution of the first antibiotic [34]. The results obtained in this study support the use of two or more antibiotics to increase the gentamicin elution from bone cement. The synergistic effect of antibiotic elution refers to the enhanced antimicrobial activity achieved by combining two or more antibiotics in bone cement or cement spacers [36]. It is a well-known phenomenon, and this is the first time it has been demonstrated in vitro using G3A bone cement [36–38]. The combination of antibiotics can synergistically affect the elution properties of the cement. When two antibiotics are present, one can enhance the release or elution of the other from the cement matrix because the combined antibiotics may change solubility or diffusion properties.

This synergistic effect can potentially improve the treatment's antimicrobial effectiveness [11,39]. By using antibiotics with different mechanisms of action, the elution properties of the cement are improved, and the likelihood of antibiotic resistance is reduced. This approach can lead to enhanced antimicrobial efficacy by targeting gram-negative and gram-positive bacteria commonly associated with prosthetic joint infections (PJI). Addition-

ally, the broadened spectrum of antimicrobial activity allows for more effective treatment against a wider range of bacterial pathogens [36,40–42].

The study has several limitations that should be considered. Firstly, it was conducted in vitro, which may not reflect real-life clinical conditions. Additionally, the study had a relatively small sample size, limiting the statistical power and generalization of the findings. It focused on a specific antibiotic combination and did not explore other combinations or individual antibiotics. In addition, it would have been of great value to have measured the elution concentrations of vancomycin and added them to the results. The evaluation of elution was limited to short-term intervals, and long-term effects were not assessed. Finally, the measurement methodology used for elution could have introduced potential measurement errors. Another limitation of this study was the use of ethylene oxide for the sterilization of the spacers. This can accelerate the chemical degradation of the gentamicin by creating an oxidizing environment, affecting its elution properties, which in the end can lead to a possible bias in our results. These limitations highlight the need for further research to address these aspects and provide a more substantial evidence base.

5. Conclusions

The conclusions of this study suggest that the timing of antibiotic addition in bone cement can influence the elution properties of gentamicin. The study found that immediate elution of gentamicin from premixed cement/antibiotic spacers (Spacer #1) resulted in significantly higher elution than spacers that underwent storage for two months (Spacer #3). Furthermore, adding antibiotic powder during spacer preparation (Spacer #2) increased elution compared to premixed cement/antibiotic spacers. In addition, this study showed that adding vancomycin to the bone cement (Spacer #4) significantly increased the cumulative gentamicin elution compared to Spacer #1. These findings suggest that the timing and method of antibiotic addition can affect the elution properties of antibiotics from bone cement spacers. However, it is essential to note that the study was conducted in vitro, and further research is needed to validate these findings and evaluate their clinical implications.

Author Contributions: Conceptualization, G.C. and D.G.; methodology, L.S. and F.B.; software, P.C.; validation, F.B., F.G. and A.M.; formal analysis, L.S. and F.D.M.; investigation, S.A. and A.B.; resources, G.C., F.B. and F.G.; data curation, G.C., F.B. and F.G.; writing—original draft preparation, D.G., G.C. and P.C.; writing—review and editing, F.B. and F.G.; visualization, F.G., L.S., P.C. and A.M.; supervision, A.M. and P.C.; project administration, G.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All procedures involving human participants' studies followed the institutional and national research committee's ethical standards and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare no conflict of interest.

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