Review

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Antibiotic Release and Mechanical Performance of PMMA Bone Cement: Findings from In Vitro Studies

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Abstract: Using polymethylmethacrylate (PMMA) bone cement is widespread in orthopedic surgeries for implant fixation and as a carrier for antibiotic delivery to prevent and treat infections. This review comprehensively evaluates in vitro studies investigating the antibiotic elution profiles and mechanical properties of PMMA bone cement. We explore the kinetics of antibiotic release, factors influencing elution efficiency, and the impact of various antibiotics on the mechanical properties of PMMA composites. At the same time, we examine how the inclusion of antibiotics affects the mechanical integrity of PMMA, including parameters such as compressive strength, tensile strength, and fatigue resistance. Through a detailed analysis of these studies, this review aims to provide insights into optimizing PMMA bone cement formulations for enhanced therapeutic efficacy and structural performance.

Keywords: Polymethylmethacrylate; PMMA; bone cement; antibiotics; elution; mechanical; in vitro; additive; porogen; nanocomposite; gentamicin; vancomycin

Introduction

Polymethylmethacrylate (PMMA) bone cement is the gold standard in orthopedic surgery since its introduction in the 1960s by Sir John Charnley [1]. Primarily used for implant fixation, PMMA's role has expanded to include acting as a delivery vehicle for antibiotics, which is particularly critical in preventing and treating infections [2]. In the context of musculoskeletal infections, research on new antibiotic carriers and their application is still crucial. The most frequent causes of revision surgery are instability, mechanical failure and periprosthetic infections [3]. For this reason, the mechanical characteristics and the elution of antibiotics from bone cement are critical for the outcome of arthroplasty procedures and load-bearing applications.

The performance of PMMA bone cement in load-bearing applications depends on its mechanical properties, which include stiffness, flexural modulus, and compressive strength. These properties are dependent on a variety of factors, including molecular weight, the ratio of polymers to monomers, grain size, antibiotic elution, defects, and voids [4]. Research has shown that the addition of antibiotics can alter these properties, potentially compromising the cement's structural integrity. For example, increasing the concentration of certain antibiotics may reduce PMMA's compressive strength, which could have an impact on the implant's longevity and dependability [5].

Antibiotic-impregnated bone cement is frequently used as a vehicle for the direct release of antibiotics at the target site, particularly in arthroplasty surgeries. Since resistant bacteria are often the source of periprosthetic joint infections (PJIs), it is important to achieve high concentrations of antibiotics at the site of infection as early as possible. As a result, the type, concentration, cement

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formulation, and polymerization process all affect the elution profiles [6]. Optimal antibiotic release is essential to guaranteeing that the local concentration stays above the minimum inhibitory concentration (MIC) for pathogens commonly associated with PJIs.

Numerous studies have investigated the biomechanical characteristics of PMMA when combined with antibiotics [7–12], but direct comparisons between studies are challenging. The optimization of PMMA formulations to attain a synergistic balance between mechanical robustness and antibiotic elution efficiency still presents obstacles, despite notable advancements. In order to improve antibiotic release while preserving mechanical integrity, current research has focused on altering the composition of PMMA, adding different types of antibiotics, and exploring novel methods and techniques to enhance drug release while maintaining mechanical integrity. This review provides an overview of the current in vitro findings, identify key factors influencing antibiotic elution and mechanical properties.

Search Strategy

Authors conducted a systematic electronic search of the PubMed database from January 2014 until May 2024, using the search criteria: ("PMMA") AND ("antibiotic"), ("bone cement") AND ("antibiotic"), or ("Polymethylmethacrylate") AND ("antibiotic"). Only studies that performed in vitro and reported either an antibiotic elution analysis or a biomechanical testing or both were included. We also included studies written in English and their full text was available. We excluded literature reviews, clinical trials, observational studies, and case studies. Moreover, we omitted studies that report dental-related applications of PMMA.

Results

The search yielded 176 results. To determine their eligibility, we examined the titles and abstracts of the retrieved studies. After applying the inclusion criteria, we found 56 relevant studies and included them in the review. Among them, 47 studies determined the antibiotic elution properties and 45 studies the mechanical properties of the formulations. In particular, 12 studies analyzed the elution profile of gentamicin alone [13–24], 11 studies vancomycin alone [9,25–34], 5 studies the combination of gentamicin and vancomycin [10,35–38], 1 study ciprofloxacin [39], 1 study the combination of ciprofloxacin and vancomycin [40], 2 studies the combination of tobramycin and vancomycin [11,41], 1 study the combination of oritavancin and vancomycin [42], 1 study the combination of ceftaroline and vancomycin [43], 2 studies the combination of daptomycin, gentamicin and vancomycin [44,45], 1 study of tobramycin, gentamicin and vancomycin [20], 2 studies teicoplanin [46,47], 1 study kanamycin [48], and the rest of the other combinations of antibiotics [8,12,49–52]. Also, researchers used several commercially available PMMA bone cement, which are presented in Table 1.

Table 1. A List of commercially available bone cement used in the reported studies and the antibiotics were incorporated into it.

Bone cement	Antibiotics loaded
Copal G	vancomycin [44,53]
	gentamicin and vancomycin [35,36,38,54]
Copal G + C	gentamicin [24]
Copal G + V	gentamicin and vancomycin [36,37,54]
Palacos R + G	gentamicin [14,17,18,24,44,55]
	vancomycin [35,53]
	gentamicin and vancomycin [36–38,54]
Palacos R	gentamicin [19,24]
	telavancin [56]
	clindamycin [57]
	vancomycin and tobramycin [11]

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	vancomycin and cefazolin[8]
	vancomycin and ciprofloxacin [40]
Palacos MV	vancomycin [28]
Palacos LV	vancomycin [30]
	meropenem [12]
	vancomycin, daptomycin, and tobramycin [45]
Simplex P	gentamicin [15,20,31,58]
	gentamicin and vancomycin [10]
	gentamicin, piperacillin, and tazobactam [50]
	vancomycin [9,27,32]
	vancomycin and tobramycin [41,59]
	vancomycin, daptomycin, and tobramycin [45]
	vancomycin and ciprofloxacin [40]
	oritavancin and vancomycin [42]
Simplex HW	gentamicin, rifampicin, and tobramycin [60]
Mendec Spine Resin	gentamicin [13]
CMW Smartset-GHV,	gentamicin [20]
Smartset-HV	vancomycin [25,33,34]
	vancomycin and ceftaroline [43]
CMW 3	kanamycin [48]
Cemex	gentamicin [23]
	vancomycin [26]
	teicoplanin [46]
	multiple antibiotics [49]
Biomet	vancomycin [29]
	vancomycin, daptomycin, and tobramycin [45]
Lima CMT1	vancomycin and ciprofloxacin [40]
G3 Low Viscosity	gentamicin, vancomycin, tobramycin [61]
Zimmer radiopaque	vancomycin, daptomycin, and tobramycin [45]
BioMedtrix	cefazolin, gentamicin, and vancomycin [62]
FIX1® Radiopaque	ciprofloxacin [39],
	ciprofloxacin and vancomycin [63]
BioFix1	teicoplanin [47]

Antibiotic Choice

In the in vitro study of **Gasparini et al.** [49] authors examined the elution kinetics of 14 different high-dose PMMA bone cements incorporated with several antibiotics such as gentamicin, amikacin, vancomycin, tobramycin, streptomycin, colistin, rifampicin, ceftriaxone, tigecycline, and meropenem for 28 days. The high-dose PMMA samples showed a burst release of antibiotics in the first hour, followed by a lower elution rate. Colistin at 0.6% discontinued its elution after the first hour, tigecycline discontinued its elution after 24 hours, colistin at 2.4% formulation discontinued its elution after 7 days, and meropenem discontinued its elution after 21 days. The cement supplemented with clindamycin released greater absolute and relative antibiotic quantities (10729μg and 57%) compared with the other cements ($p<.001$ for both). Among the aminoglycosides, tobramycin exhibited higher absolute and relative elution compared to gentamicin and amikacin ($p<.001$ for each), and there were no significant differences observed when compared to streptomycin. Regarding the glycopeptides, the crystallized formulation of powder vancomycin presented higher absolute elution than the lyophilized formulation ($p<.001$ for both absolute and relative elution). Researchers also found that the crystallized formulation of vancomycin had a higher absolute elution compared to teicoplanin ($p<0.001$). The crystallized formulation of vancomycin showed a greater absolute elution than tobramycin ($p<0.001$), when different antibiotic classes were compared. Clindamycin exhibited significantly higher absolute and relative elution compared to colistin at 2.4%, rifampicin,

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ceftriaxone, tigecycline, and meropenem ($p<0.001$ for each). The authors concluded that this in vitro study demonstrates theoretical advantages in the preparation of antibiotic-loaded acrylic cement for some antibiotics not routinely used in the clinical setting for PJIs.

To achieve effective infection control, it is essential to control the release of antibiotics from PMMA. However, the elution rates of antibiotics are low, and there is a lack of understanding regarding the mechanisms involved. Thus, **Paz et al.** [8] assessed whether the presence of vancomycin and/or cefazolin could affect cement behavior. Six groups with antibiotic levels ranging from 2.5 to 10wt.%. were studied. Group 1 was the control group without antibiotic. For group 2, either vancomycin or cefazolin were added up to 2.5 wt.%. In groups 3, 5 and 6, only vancomycin was added (2.5, 5 and 10 wt.%, respectively), and in group 4 both cefazolin and vancomycin (2.5 and 5 wt.%, respectively). Groups with cefazolin (G2, G4) showed significantly greater elution than those containing the same concentration of vancomycin even after 1 month greater ($p < 0.001$ in all cases). Moreover, groups containing the same amount of antibiotic (2.5 wt.%) showed significant differences ($p < 0.01$) depending on the type of antibiotic. Regarding the compressive strength, group 2 with 2.5wt.% cefazolin, group 4 with both antibiotics and group 6 experienced significant reductions in compressive properties after immersion ($p < 0.01$). While the bending strength of each group without aging showed a slight decrease as compared to the control cement, after aging, this decreased significantly in the groups with cefazolin (G2 and G4) ($p <0.01$). Analysis of SEM images displayed differences in the size and morphology of both antibiotics. **In conclusion**, groups with cefazolin showed much higher elution than those containing the same concentration of vancomycin. In contrast, groups with cefazolin showed a lower strength than vancomycin groups.

Ficklin et al. [62] evaluated the effects of the addition of antibiotic drugs (such as gentamicin, vancomycin, and cefazolin) and silver on the compressive and bending strength of PMMA. Doses of 0.5g, 1g, 2g, or 3g of each antibiotic powder and 0.25 g of silver microparticles were added to the PMMA powder. All test groups had a significantly inferior compressive strength than the control plain PMMA, except for the 2g cefazolin group, which was not significantly lower. The 0.5g vancomycin group was significantly stronger than the 0.5 g gentamicin group. Groups with 1g, 2g, and 3g of cefazolin were also significantly stronger than their counterparts containing comparable quantities of vancomycin or gentamicin. On the other hand, all test groups containing silver microparticles were considerably weaker than the control group. For instance, the group containing 0.5g vancomycin + silver was notably weaker than the silver + PMMA group. The compressive strength of all groups, except for the groups with 3g vancomycin, 2g gentamicin, 3g gentamicin, and 0.5g vancomycin + silver, was significantly greater than the ASTM minimum standard of 70 MPa. The addition of silver to 0.5g vancomycin and 1 g cefazolin resulted in a significant decrease in bending strength compared to the silver + PMMA group, but otherwise the addition of silver did not significantly affect bending strength. Only the group of 3g vancomycin was substantially weaker than the ISO minimum standard for bending strength (50 MPa). In conclusion, the addition of antibiotic or silver decreased the biomechanical strength in all samples, but not below the ASTM or ISO standard for most groups. Adding cefazolin appears to affect strength the least, while high doses of vancomycin alter strength the most.

Frew et al. [35] investigated whether the elution of vancomycin from "homemade'' cement was comparable with more expensive commercially available vancomycin-impregnated cement. Three groups of cement were prepared, group 1 (commercially prepared cement- Copal G + V) containing 0.5g gentamicin and 2g vancomycin, group 2 ("home-made", manufacturer mixed cement) Palacos R+G + 2 g of vancomycin powder added by gradually combining equal volumes of antibiotic and cement powder and repeating until all antibiotic is mixed into the powder, and group 3 ("home-made", ad hoc mixed cement) Palacos R+G + 2 g of vancomycin powder mixed following a method similar to that used in the operating theatre. The elution of gentamicin and vancomycin from the "ad hoc" preparations (group 3) was significantly higher than other groups ($p = 1.56 \times 10^{-4}$ and $p = 2.02 \times 10^{-5}$, respectively). The mean peak concentration of gentamicin was 756 μg/ml and the mean peak concentration of vancomycin was 677 μg/ml (475 to 1028) with the 'ad hoc' mixed cement. When vancomycin was added to Palacos R+G cement in group 2, the results were comparable with

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commercially prepared cement (group 1), with mean concentrations of vancomycin ranging from 68 μg/ml to 149 μg/ml and concentrations of gentamicin ranging from 301 μg/ml to 471 μg/ml. The authors concluded that they found no significant advantages of using expensive commercially produced vancomycin-impregnated cement and recommended the addition of vancomycin powder by hand in the operating theatre.

Cacciola et al. [61] evaluated the mechanical and elution properties of G3 Low Viscosity Bone Cement loaded with various doses of up to three antibiotics. Twelve specimens were prepared with diverse doses of gentamicin (2g and 4g), vancomycin (2g, 3g, 4g, and 6g), and tobramycin (2g, 3g, 4g, and 5g). The authors only measured the vancomycin elution across the specimens. For all the specimens, the vancomycin release was fast in the first 72 hours (mean ratio 65.6%, range 48.2–91.2%), showing a small reduction in the following days. The highest elution of vancomycin was observed in the 2g specimen consisting of 2g vancomycin, 2g tobramycin, and 4g gentamicin, respectively while for the 4g specimen, the highest elution was observed in the specimen of 4g vancomycin, 2g tobramycin, and 4g gentamicin, respectively. Adding one more ($p < 0.05$) or two ($p < 0.05$) antibiotics showed a statistically significant increase in vancomycin elution. The mean compressive strength of the twelve specimens was 82 MPa (range 111 to 67 MPa), ten out of twelve specimens reached the minimum level suggested by ISO 5833, and only specimen 10 (total 8g of antibiotics: 4g of vancomycin and 4g tobramycin) and specimen 12 (total 10g of antibiotics: 4g of vancomycin, 4g of tobramycin and 3 g of gentamicin) didn't reach the minimum threshold of 70 MPa (respectively, 69.1 MPa and 66.8 MPa). The increase in antibiotics dose confirmed the decrease in compressive strength, highlighting the impact of a large quantity of antibiotics on the mechanical properties of bone cement. The mean bending strength and bending modulus were respectively 2162MPa (range 1920 to 2439 MPa) and 37 MPa (range 28–47 MPa). By adding a second or a third antibiotic, the compressive strength of 2g or 4g vancomycin-loaded cement decreases, but the reduction is not statistically significant when a third antibiotic is added. The addition of antibiotics to the cement does not influence the bending Modulus, and only in a few cases, its value showed statistically significant variation. **According to this study** mechanical properties do not decrease significantly by adding large doses of antibiotics, or up to three antibiotics, while the vancomycin elution increases until swelled to twice.

In this study of **Slane et al.** [11], authors investigated the influence of dual antibiotic loading on the total antibiotic elution and compressive mechanical properties of acrylic bone cement. Multiple concentrations of vancomycin (V) (0–3g) and tobramycin (T) (0–3g) were added either alone or in combination with PMMA, resulting in 12 experimental groups. The PMMA group T3V2 (2g of vancomycin and 3g of tobramycin) eluted the highest cumulative concentration of both antibiotics, with 2.41mg of vancomycin and 2.95mg tobramycin, respectively relative to any other cement group ($p<0.001$). For cement containing only a single antibiotic, the cumulative elution of tobramycin was always greater compared to vancomycin. For groups containing the same concentration of antibiotic, the elution of tobramycin was constantly higher. All cement groups exhibited a burst effect during the first 24h following however cements containing lower concentrations of antibiotics, tended to flat after the initial burst release. The cumulative elution profiles of tobramycin primarily showed a substantial synergistic effect, as group T3V0 (3g of tobramycin) released 1.08mg over 28 days, and the incorporation of 1g of vancomycin resulted in an approximately 38% increase in the elution of tobramycin. The compressive modulus and compressive strength of the bone cements were significantly affected by the inclusion of antibiotics. Most cement groups presented a significantly lower compressive modulus relative to the control cement($p<0.05$). The largest reduction seen in modulus was for group T3V2 (1,084 MPa), which had a 37% reduction relative to the control cement. Regarding compressive strength, most cements were also significantly lower than the control cement and several cements were below the requirement established in ISO 5833. All cements containing antibiotics, regardless of the loading ratio, had significantly higher porosity relative to the control cement**. In conclusion**, this study demonstrates that high antibiotic loading in cement does not necessarily lead to enhanced antibiotic elution while regardless of the loading ratio, cements

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containing antibiotics showed a significant decrease in the mechanical properties and an increased porosity. Furthermore, tobramycin elutes more effectively than vancomycin from cement.

Boelch et al. [38] compared the antibiotic elution and the compressive strength of Copal spacem when gentamicin and vancomycin were added (COP specimens) to those properties for Palacos R+G when vancomycin was added (PAL specimens). In total, 6 specimens were prepared, 3 specimens of Palacos R+G COP2 with 0.5g gentamicin and 2g vancomycin, COP4 with 0.5g gentamicin and 4g vancomycin, and COP6 with 0.5g gentamicin and 6g vancomycin, and 3 specimens of Copal PAL2 with 0.5g gentamicin and 2g vancomycin, PAL4 with 0.5g gentamicin and 4g vancomycin, and PAL6 with 0.5g gentamicin and 6g vancomycin. The COP specimens produced significantly lower cumulative gentamicin concentrations than the PAL specimens at each of the 9 time points of the study ($p \leq 0.005$). The COP2 specimen produced much higher ($p \leq 0.043$) cumulative vancomycin concentrations after day 2. For the COP4 specimens, significantly higher ($p \leq 0.035$) cumulative vancomycin concentrations were measured after day 1, and for the COP6 specimens at every measurement ($p \leq 0.004$). Apart from PAL4, compressive strengths before the elution testing were below the acceptable 70 MPa level set by ISO 5883. Following the elution tests, COP2's compressive strength was considerably less than PAL2's ($p = 0.005$). When the antibiotics were eluted, the compressive strength of the COP and PAL specimens loaded with 4 g and 6 g of vancomycin significantly decreased ($p \leq 0.014$). **This study** did not demonstrate consistent superior antibiotic elution from Copal® spacem compared to Palacos® R+G for fabricating gentamicin and vancomycin-loaded spacers.

In their following study, **Boelch et al.** [37] investigated the effect of eluate volume change on antibiotic elution and mechanical properties from different vancomycin (COPAL G + V as COPV, PALACOS R + G +2g vancomycin as PALV) and gentamicin (PALACOS R + G as PALG) loaded bone cement. From 6 hours on, most formulations yielded noticeably reduced gentamicin concentrations in 8 ml compared to 4 ml. At six weeks, every concentration of PALG in eight milliliters was below the lower measurement limit. For both PALV and COPV, the vancomycin concentrations were considerably lower in 8 ml than in 4 ml starting at 6 hours. Vancomycin concentrations for PALV fell below the lowest measurement limit in 8 ml on day 10 and in 4 ml on week 3. For COPV, concentrations fell below the lowest measurement limit in 8 ml on day 7 and 4 ml on week 2. PALV and COPV had significantly lower yield strengths after immersion in 4 ml ($p = 0.020$ and $p = 0.007$) and in 8 ml ($p < .000$ each) compared to PALG. Within the formulations, doubling eluate volume reduced yield strength significantly for PALV ($p = 0.011$) and COPV ($p = 0.006$). **Thus, eluate volume change influences** antibiotic elution depending on the antibiotic combination and loading technique. The reducing effect is higher on vancomycin than on gentamicin elution. The compressive strength of gentamicin/vancomycin-loaded bone cement after immersion is eluate volume dependent.

In the study of **Lee et al.** [48], authors investigated the elution of kanamycin as an antibiotic-loaded bone cement and the mechanical strength of kanamycin-loaded cement compared with vancomycin-loaded cement. For the elution test, 3 doses of kanamycin (1g, 2g, or 3g) were mixed with bone cement powder. For the ultimate compression strength (UCS) testing, 3 different doses of kanamycin (1g, 2g, or 3g) and 2 doses of vancomycin (1g and 2g) were mixed with bone cement powder. Kanamycin has been detected in eluates of all regiments during the 30-day eluting period. Concentrations of all antibiotics had been decreased with time. However, regardless of the initial dose mixed, there was no difference in the amount of elution at day 30 (1 g of kanamycin, 3.07 ± 0.42 mg/mL; 2 g of kanamycin, 4.04 ± 1.14 mg/mL; 3 g of kanamycin, 5.11 ± 2.27 mg/mL; P =0.372). The UCS values of the plain cement were 99 MPa and 96 MPa before and after the elution test, respectively. With more antibiotics included in the cement, the pre-eluted compression strength of cement loaded with kanamycin and vancomycin was lower (1g of kanamycin, 97 MPa; 2 g of kanamycin, 97 MPa; 3g of kanamycin, 95 MPa; 1 g of vancomycin, 96 MPa; and 2g of vancomycin, 95 MPa). After 30 days of elution, the strength of each plain, kanamycin-loaded, and vancomycin-loaded cement was significantly lowered than that of the initial specimens ($p < .05$**).** According to this study, the antimycobacterial activity of antibiotic-loaded bone cement containing more than 2 g of kanamycin was effective during a 30-day period and the ultimate compression strength of bone

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cement loaded with 1-3 g of kanamycin was comparable with 1 g of vancomycin while maintaining effective elution until day 30.

Haseed et al. [43] tested the ability of ceftaroline to serve as a local antibiotic embedded in PMMA. For this purpose, 3 groups of ceftaroline 0.6g (1.5 wt.%), 1.2g (3 wt.%) and 1.8g (4.5 wt.%), and 3 groups of vancomycin 1g (2.5 wt.%), 2g (5 wt.%) and 3 g (7.5 wt.%) were used. Ceftaroline at 1.5 wt.% was released up to 3 weeks above MIC and the following two weeks just below MIC. Ceftaroline at 3 wt.% eluted up to the sixth week above the MIC and 7.5 wt.% eluted up to the seventh week above MIC. Vancomycin at 2.5 wt.% elutes up to 3 weeks with the same concentration as MIC, while both 5 wt.% and 7.5 wt.% released at or above MIC for 5 weeks, respectively. Regarding three-point bending, between 1.5-wt.% (43 N, $p = 0.098$) and 3-wt.% (42N, $p = 0.065$) of ceftaroline, there was no significant drop in strength. However, at 4.5-wt.% (37N, $p < 0.001$) ceftaroline, authors noticed a significant drop. For vancomycin, there was a significant decline in strength at 5 wt.% (42N, $p = 0.02$) and 7.5 wt.% (35N, $p < 0.001$), respectively. Regarding axial loading (compression), at 4.5 wt.% (42N, $p < 0.001$) ceftaroline, there was a significant drop in the strength of PMMA. For vancomycin, there was a significant drop in the strength in groups of 5 wt.% and 7.5wt%, respectively ($p < 0.001$). In the ceftaroline group, authors reported a significant decline in stiffness upon the addition of 1.5 wt.% of antibiotic, 22N ($p = 0.01$), 3 wt.% of ceftaroline, 20N ($p < 0.001$) and 4.5 wt.% of ceftaroline, 19N ($p < 0.01$). In a similar way, upon addition of higher amounts of vancomycin there was a significant drop in stiffness, 19N ($p < 0.001$) for 2.5-wt.% vancomycin), 19N ($p < 0.001$) for 5 wt.% vancomycin and 18N ($p < 0.001$) for 7.5 wt.% vancomycin. According to this study, ceftaroline- a cephalosporin that is effective against methicillin-resistant Staphylococcus aureus (MRSA) infections- loaded at similar concentrations as vancomycin into PMMA, is a more potent alternative based on its more favorable bioactivity and elution properties, while having a lesser effect on the mechanical properties of the cement.

Ajit Singh et al. [59] assessed the mechanical strength of hand-mixed vancomycin bone cement at different concentrations with commonly used industrial pre-blended antibiotic bone cement. Three groups of samples were prepared, plain PMMA, vancomycin-PMMA (consisting of 1g, 2g, 3g, or 4g of vancomycin), and commercially available tobramycin-PMMA. The mean three-point bending of plain PMMA, vancomycin-PMMA, and tobramycin-PMMA revealed significant differences between 2g vancomycin-PMMA (1.71 kN, $p = 0.016$), 3g vancomycin-PMMA (1.30 kN, $p = 0.0006$), and 4g vancomycin (1.27 kN, $p = 0.0004$). There were no significant differences between the plain PMMA and the different vancomycin-PMMA samples in stiffness**.** It was concluded that hand-mixed antibiotic cement (HMAC) is advantageous as a cement spacer but it is not recommended for primary arthroplasty and second-stage revision arthroplasty as it showed variable mechanical strength varying on the concentration of antibiotics used and therefore the industrial preblended antibiotic cement is superior to hand-mixed cement. Furthermore, the recommended maximum concentration of vancomycin based on this study is 2 g/pack (40 g) of cement.

In their study, **Schmidt-Malan et al.** [42] tested whether 7.5% w/w oritavancin- a long half-life lipoglycopeptide with broad activity against Gram-positive bacteria- mixed into PMMA affects cement strength and its elution ability, compared with vancomycin. The researchers prepared study samples in three ways to determine the most effective method for preventing oritavancin from binding to the mold surface. Also, plain PMMA and PMMA with 7.5% vancomycin were prepared. The maximum concentration of oritavancin was 1.7 μg/ml in 2 h, while for vancomycin was 21.4 μg/ml at the same time point. The mean 24-hour cumulative percent elution of oritavancin was 1.6% compared to 9.4% for vancomycin. Regarding the mechanical properties of the samples, the median 2% offset compressive strengths for plain PMMA among days 0, 3, and 7 was 80, 93, and 98 MPa, respectively whereas for PMMA with oritavancin, 79, 86, and 83 MPa, respectively. The median 2% offset compressive strengths of PMMA with vancomycin were 72, 65, and 66 MPa, respectively. The compressive elastic modulus of plain PMMA on days 0, 3, and 7 was 1226, 1299, and 1394 MPa, respectively, and the compressive elastic modulus of PMMA with oritavancin was 1253, 1078, and 1245 MPa, respectively. On the other hand, the compressive elastic modulus of PMMA with vancomycin was 986, 879, and 779MPa, respectively. According to this study, oritavancin-loaded

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PMMA had higher compressive strength than vancomycin-loaded PMMA on days 3 and 7 and higher compressive elastic moduli than vancomycin-loaded PMMA on days 0 and 7. However, proportionally less oritavancin than vancomycin eluted out of PMMA.

Meeker et al. [45] assessed the elution of properties of vancomycin, daptomycin, and tobramycin from four commercially available PMMAs (Palacos LV, Simplex P, BIOMET, and Zimmer Biomet). 1g of vancomycin or 500 mg of daptomycin or 1.2 g of tobramycin were loaded in each PMMA cement. Regarding the vancomycin elution profile, Palacos release was significantly higher than those of all other cements tested ($p < 0.00001$ for all comparisons), while the elution profile of Simplex was much lower than those of all other cements ($p < 0.001$). The Daptomycin elution profile showed that release from Simplex was significantly lower than those for the other formulations tested ($p < 0.00001$), and the elution profile from Zimmer was lower than the profiles from Cobalt and Palacos ($p < 0.001$). In the elution of tobramycin, the only significant difference observed was a significantly lower elution profile from Simplex by comparison to the other formulations tested ($p < .00001$). In conclusion, Simplex P exhibits a significantly lower elution profile than all other cements tested. In general, Palacos LV exhibits an increased elution profile compared with other cements.

Kim et al. [27] studied the effect of five different loading masses (0.125g, 0.25g, 0.5g, 1.0g, and 2.0g) of vancomycin on the mechanical properties of PMMA bone cement (Simplex™ P) and antibiotic release profile. All samples displayed a burst of cumulative elution of vancomycin within a week and 1.5%-2.6% of antibiotic eluted over the 60 days. In PMMA samples of lower antibiotic amounts (0.125g, 0.25g, and 0.5g, the elution profile tended to be zero after the initial burst. The group of 2g of added vancomycin revealed the most vancomycin eluted per cement disk. The average flexural modulus for each group was above the ISO minimum requirement (1800 MPa). Compared to the control group (mean 63 MPa), all treatment groups exhibited significantly lower flexural strength. The compressive modulus of formulated bone cement was not significantly affected by added vancomycin as compared to the control group (mean 1694 MPa) with the exception of the 2g of added antibiotic group. Similarly, added vancomycin did not significantly change the compressive yield strength as compared to the control group (81 MPa) except for the 2 g of added antibiotic group. This study did not find an ideal amount of vancomycin added to Simplex™ P that meets both strength and antibacterial requirements.

Karaglani et al. [24] investigated the elution profile of gentamicin from commercially available and "home-made" PMMA preparations. In specific, three commercially available premixed PMMA bone cements (PALACOS® R + G, COPAL® G + V, and COPAL® G + C as Groups B, C, and D, respectively) were used and three ad hoc mixed "home-made" PMMA cements (Groups F-G) were prepared similarly to the commercials. All cement beads had high initial elution during the first hour which then slowly decreased. Group B showed significantly higher gentamicin elution compared to the "home-made" Group F cement which was kept almost double for 24. Group C showed slightly better gentamicin elution profiles than the "home-made" Group G at all seven time points but these differences were not statistically significant. Group D showed a superior gentamicin elution profile compared to the "home-made" Group H. The results of this study suggest that adding gentamicin manually to PMMA cement in the operating theater produces on the one hand inferior antibiotic elution at concentrations of 1.2% and 2.4% (without the presence of vancomycin) but on the other hand, superior antibiotic elution when vancomycin is present to the mix.

In the preliminary study of **Gandomkarzadeh et al.** [63], authors examined the effects of ciprofloxacin and vancomycin on the mechanical properties of PMMA bone cement. 6 groups of PMMA plus antibiotic were prepared containing 2.5%, 5%, and 10% w/w of each antibiotic separately and plain PMMA was used as a control. After day 1, the compressive strength in the presence of 2.5%, 5%, and 10% of ciprofloxacin significantly decreased to 5%, 13% and 14%, respectively ($p < 0.001$). Impregnation of 2.5%, 5%, and 10% of vancomycin led to decreases equal to 7%, 15%, and 35.5%, respectively, on day 14. Nevertheless, the cement containing 2.5% of both antibiotics showed acceptable compressive strength according to ISO5833 standard level (<70 MPa) over 28 days of the experiment. The flexural strength reduction in the cement containing 2.5% ciprofloxacin and vancomycin was equal to 13% and 13.5% on days 14 and 9 and 9.5% on day 28, respectively. Flexural

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modulus reduction in cements containing 2.5%, 5%, and 10% of ciprofloxacin was equal to 6.5%, 17%, and 21%, respectively, on day 14 and was equal to 6.5%, 10%, and 14%, after 28 days (Figure 2a). In the group of vancomycin, the flexural modulus of the cement containing 2.5%, 5%, and 10% antibiotic decreased to 5%, 18%, and 33%, respectively, on day 14, while these values increased on day 28. The porosity of dry and wet bone cement was increased to 3.05 and 3.67% by the addition of ciprofloxacin and vancomycin to the cement, respectively ($p < 0.001$). following 14 days of immersion with large pores of approximately 135μm in average. In conclusion, the effect of antibiotic loading is both molecular weight and drug content dependent. The time is also an important parameter, and the second week is the probably optimum time to study mechanical behavior of antibiotic-loaded bone cement.

Following on their next study, **Gandomkarzadeh et al.** [39] evaluated the effect of ciprofloxacin concentration and cement geometry on release and mechanical properties of PMMA bone cement. For this study, three different formulations with geometries of circular slab, rectangular prism and short cylinder were made. Ciprofloxacin was added again in concentrations of 2.5%, 5% and 10% w/w in the cement. Release profiles of the three cement geometries containing 2.5%, 5% and 10% antibiotic showed a two-phase behavior, a high release rate that ends by a plateau and after the plateau, the release rate started to increase again. The total amounts of released drugs in different geometries over 28 days were 14-19%, 16-20% and 24-36% of the loaded drug for drug contents of 2.5, 5.0 and 10.0%, respectively. Results showed that in all systems compression strength decreased by time, but in 2.5% antibiotic formulation the strength was in the acceptable range (≥ 70 MPa). On the other hand, in formulations containing 5% and 10% of ciprofloxacin, compression strength decreased by about 11% and 21% by day 7, respectively, in comparison to the control group ($P < 0.05$) and decreased to an amount lower than 70 MPa in later times. In conclusion, the results of antibiotic-loaded bone cement tested for cement strength, drug release behavior, and antibacterial activity are affected by prepared as slab, rectangular prism, and short cylinder and geometry (cement prepared as slab, rectangular prism, and short cylinder).

The research group of **Morejón Alonso et al.** [52] prepared PMMA cements loaded with 10 wt.% of the drug Oleozon and mixtures of Ciprofloxacin/Meropenem and Ciprofloxacin/Meropenem/Oleozon to investigate in vitro elution release profiles. All formulations revealed an initial burst antibiotic, and afterward, the elution rate declined to maintain a sustained release pattern over time. Because of its oily nature, Oleozon is released at a lower rate and in a smaller amount compared to Ciprofloxacin or Meropenem drugs. Meropenem's initial release was more rapid than Ciprofloxacin's, but on day 11, the release of Ciprofloxacin was statistically higher. The presence of Oleozon decreased the Ciprofloxacin elution rate more than that of Meropenem. On the other hand, the release of Oleozon was slightly increased by the presence of the two other hydrophilic drugs. The results indicated a positive antibacterial effect by the combined use of the two or the three drugs tested against the Gram-negative bacilli Pseudomonas *aeruginosa*.

Gentamicin is an antibiotic that is commonly used in combination with PMMA; however, gentamicin powder is hard to find in many countries. Thus, **Liawrungrueang et al**. [19] evaluated the elution characteristics of gentamicin-impregnated PMMA made with lyophilized liquid gentamicin (LG) compared with gentamicin-impregnated PMMA made from gentamicin powder (PG). Eluates from both groups had high concentrations of gentamicin on day 1 (113.63 ± 23.42 mg/dl in LG-PMMA and 61.7 ±8.37 mg/dl in PG-PMMA) and experienced a continuous decrease for up to 6 weeks (3.28 ± 1.17 mg/dl in LG-PMMA and 1.21 ± 0.28 mg/dl in PG-PMMA). Throughout the experiment, LG-PMMA presented significantly higher levels of gentamicin concentrations compared to the PG-PMMA group at all time points ($P< 0.05$). Scanning electron microscope (SEM) evaluation pointed out that the surface area of the LG-PMMA was more porous than the PG-PMMA spacers. In conclusion, gentamicin-impregnated PMMA made with lyophilized liquid gentamicin had approximately a two times higher rate of antibiotic elution in preliminary in vitro studies, as compared with PMMA made with premixed gentamicin powder.

Chen et al. [22] investigated the effects of different cement formulations i) liquid/powder (LP) ratios (70%, 85%, 100%, and 115%), ii) ratios of $BaSO_4$ as radiopacifier (10%, 15%, 20%, 25%, and 30%),

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iii) rations of BPO as initiator (0.5%, 1%, 1.5%, 2%, and 2.5%) and iv) doses of gentamicin (0.05g, 0.1g, 0.2g, 0.3g, and 0.4g) on the porosity, gentamicin elution rates for 28 days and mechanical properties of PMMA. The porosity of LP70 was 72.3% which was much higher than other groups (12~19.6%). LP70 showed the lowest compression strength of 42.3 MPa, which was below the ISO standards (70 MPa), while LP85, LP100, and LP115 exhibited better compression strength by exceeding 70 MPa. With the increased ratio of BaSO4 added to the cement, the porosity and pore diameter increased. Bone cement with lower ratios of radiopacifier (R10, R15, and R20) had a smooth surface, while an increased ratio (R25 and R30) resulted in a rough surface. Bone cement with 1.5% BPO (I1.5) displayed the lowest porosity and pore diameter. As the added concentration of gentamicin increased, cement porosity and pore diameter increased. All cements showed burst release of the antibiotic during the first day of elution, and the elution rate decreased to sustain a constant drug release over time. Among the groups of different liquid/powder ratios, LP70 demonstrated the best cumulative elution of about 73.8% at day 28 and the gentamicin release decreased with the increased liquid/powder ratio (LP85 vs. LP100: 31.2% vs. 13%), though LP100 showed similar gentamicin release behavior with LP115 (LP100 vs. LP115: 13% vs. 15.7%). A higher ratio of radiopacifier (R) enhanced the elution rate and the cumulative release of gentamicin from the bone cement as the percentage of the cumulative release of gentamicin was 13%, 14.6%, 21.9%, 24%, 24.7% for R10, R15, R20, R25, and R30, respectively. The gentamicin elution profile and cumulative release data suggested that the ratio of the initiator exerted no significant effects on gentamicin release (cumulative release percentage: 20.4%, 18.1%, 13%, 21.1%, 15.1% for I0.5, I1, I2, I2.5, respectively). In conclusion, by varying the composition of ALBC, could considerably enhance the antibiotic elution rates by increasing porosity, while maintaining an adequate mechanical strength of the bone cements.

Wang et al. [12] investigated the biomechanical and elution properties of meropenem-loaded bone cement. For this purpose, six formulations were made: (i) bone cement without antibiotics (control, A0); (ii) bone cement with 5% meropenem (A2); (iii) bone cement with 10% meropenem (A4); (iv) bone cement with 15% meropenem (A6); (v) bone cement with 5% vancomycin (B2); and (vi) bone cement with 10% vancomycin (B4). At 24 days of immersion, the eluted meropenem concentration of samples A2, A4, A6 was respectively 0.36 μg/mL, 0.62 μg/mL and1.01 μg/mL. Meropenem was released rapidly during the first 48 hours and decreased throughout the remainder of the study period. All cements compressive strength values were above the minimum requirement of ISO 5833, except for groups B2 (69 MPa) and B4 (57 MPa). Group A4 (101 MPa) showed higher compressive strength than group A0 (93 MPa) ($p < 0.05$), but no difference was found between the A0 (93 MPa), A2 (97 MPa) and A6 (94 MPa) groups ($p > 0.05$). Again, group A4 (71 MPa) revealed higher bending strength than those of group A0 (64 MPa) ($p < 0.05$), however, there was no difference between the A0 (64 MPa), A2 (68 MPa) and A6 (65 MPa) groups ($p > 0.05$). All the bending modulus values of A0 (2402 MPa), A2 (2465 MPa), A4 (2473 MPa), and A6 (2416 MPa) groups were well above the minimum requirement of ISO 5833 but no intergroup differences were observed ($p > 0.05$). In conclusion, bone cement with 10% (4 g/40 g) meropenem had the best performance and at a constant temperature of 37°C, meropenem can be released from bone cement for up to 24 days. When adding up to 15% (6 g/40 g) meropenem to the bone cement, the biomechanical properties were not reduced.

Sophie et al. [57] examined the impact of loading different concentrations of clindamycin on PMMA cement and its mechanical properties. Two reference formulations were created, reference 1a consisting of PMMA + 1g clindamycin powder and reference 1b consisting of PMMA with gentamycin+ 1g clindamycin powder, in both formulations PMMA powder and clindamycin powder were mixed. Then, authors mixed clindamycin solution with the monomer liquid to create two test formulations. Test 2a included PMMA + 1ml clindamycin liquid, and test 2b included PMMA with gentamycin+ 1ml clindamycin. Also, two other formulations were prepared where PMMA powder was mixed with clindamycin solution, test 3a PMMA + 1ml clindamycin liquid, and test 3b PMMA with gentamycin+ 1ml clindamycin liquid. Lastly, the authors produced the test samples of group 4 by mixing clindamycin solution with the cement during the mixing procedure, including test 4a with PMMA + 1ml clindamycin liquid and test 4b with PMMA and gentamycin + 1ml clindamycin liquid. The compression strength of test groups 2a and 2b was comparable to references 1a and 1b,

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respectively, among the different test groups. Group 4a showed the biggest drop (8.8%), whereas Group 3a deviated significantly from the standard with a compression strength reduction of only 1.8%. With a 6.3% decrease from reference 1b, test 3b in group B showed the most change. Every test group that was examined had a different flexural modulus than the reference groups. Groups 2a, 3a, and 4a all displayed reductions of 5%, 13%, and 12%, respectively. Test groups 3b and 4b each displayed a reduction of 12.5%, whereas group 2b within group b demonstrated the least variation from the reference, with a reduction of 1.4%. The strength values that were established based on flexural. Every test group showed a difference in DIN impact strength when compared to the references. Test group 3a exhibited the least variation to the equivalent reference, with a 16% decrease. Test groups 2a and 4a displayed reductions of 16% and 22%, respectively. With a reduction of just 2%, test group 3b in group b was the most similar to its reference; test groups 2b and 4b displayed reductions of 11% and 5.3%, respectively. Test groups 2a (−6.9%) and 2b (0.7%) were the most similar to their respective references in terms of DIN flexural strength. Test groups 3a and 4a displayed flexural strength reductions of 17% and 13%, respectively, in comparison to reference 1a. The authors recommend the admixture of liquid antibiotic only in case powdery antibiotics cannot be used. They also recommend the admixture of liquid antibiotics to liquid cement before dough production.

In this study of **Lunz et al.** [54], authors compared the mechanical characteristics of six dual antibiotic-loaded bone cement preparations (groups A–F) made from three different PMMA bone cements to determine the effect of time and antibiotic concentration. The authors classified each bone cement as a low (2 g vancomycin) or a high (4 g vancomycin) concentration group according to the total amount of vancomycin powder per cement. Gentamicin concentration, either as premixed or as manually added, remained at 0.5g in all groups. Groups A (Copal +2g vancomycin +0.5g gentamicin) and B (Copal +4g vancomycin +0.5g gentamicin) displayed the lowest bending strength with a mean of 42 MPa and 41 MPa, respectively, while groups E (Copal G + V, 0.5g gentamicin, 2g vancomycin) and F (Copal G + V, 0.5g gentamicin, 2g+2g vancomycin,) achieved the highest results with a mean of 58 MPa and 50 MPa, respectively. Spacers of groups C (Palacos R + G 0.5g gentamicin +2g vancomycin) and D (Palacos R + G 0.5g gentamicin +4g vancomycin) showed a bending strength of 49 MPa and 47 MPa, respectively. After incubation for 6 weeks in PBS the four-point bending test was repeated in the same way. Spacers made from groups E and F showed a higher decline in bending strength with a mean bending strength of 46 ± 2 MPa and 36 MPa, respectively. Groups A and B showed a mean bending strength of 38 MPa and 31 MPa, respectively, while groups C and D showed a mean of 43 MPa and 39MPa, respectively. Two preparations (Group E and F) surpassed the minimum requirement of 50 MPa according to ISO 5833:2002 and ISO 16402:2008 after incubation for 24 h. None of the tested preparations passed the minimum requirement after incubation for six weeks. When authors examined the bending strength of the low and high-concentration preparations of the same bone cement, discovered that there were statistically significant differences between groups A and B ($p < 0.001$) and groups E and F ($p < 0.001$). Next, using 2g of vancomycin to assess all preparations, authors discovered statistically significant differences between groups A and C ($p = 0.005$) and A and E ($p < 0.001$). When all the preparations using 4g of vancomycin were analyzed, it was found a statistically significant difference between groups B and F ($p = 0.02$) and D ($p < 0.001$). The authors concluded that the intraoperative addition of 4 g of vancomycin powder per 40 g of gentamicin-premixed Palacos R + G (Group D) is mechanically the preparation of choice if a dual antibiotic-loaded bone cement spacer with high antibiotic concentrations and good stability is warranted. They also suggest that the mechanical strength of antibiotic-loaded PMMA bone cement critically decreases even over the short period of six weeks.

In another study of **Lunz et al.** [36], authors investigated the most ideal composition of a drug-eluting dual antibiotic-loaded bone cement among three different PMMA bone cements. Again, six different preparations (groups A-F) were included in the experiment. Depending on the kind of bone cement being utilized, different effects were observed in the release of gentamicin as the concentration of vancomycin increased. Spacers made of Copal (group A 28.9 mg/l and group B 26.1 mg/l; $p = 1.0$, respectively) or Copal G + V (group E 200.2 mg/l and group F 203.3 mg/l; $p = 1.0$,

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respectively) showed no effect, but spacers made of Palacos R + G (group C 149.4 mg/l and group D 226.1 mg/l; $p < 0.001$, respectively) showed a statistically significant enhancement. The average cumulative concentration of gentamicin over six weeks did not differ statistically significantly between Palacos R + G (group D) and Copal G + V (group F), at 226.1 mg/l and 203.3 mg/l, respectively ($p = 0.38$). However, there was a significant difference in the average cumulative concentration of gentamicin between groups B (26.1 mg/l) and D (226.1 mg/l) ($p < 0.001$), as well as between groups B (26.1 mg/l) and F (203.3 mg/l) ($p < 0.001$). The groups that used 40 g of bone cement and 4 g of vancomycin powder fared much better than the groups that used 2 g of vancomycin, regardless of the type of bone cement employed. The 6-week mean cumulative release of vancomycin showed significant differences between group A (49.3 mg/l) and group B (110.2 mg/l; $p < 0.001$), group C (86.2 mg/l) and group D (293.5 mg/l; $p < 0.001$), and group E (91 mg/l) and group F (251.2 mg/l; $p < 0.001$). The highest six-week mean cumulative release of vancomycin was observed in spacers of group D (293.5 mg/l). There were also significant differences in the six-week mean cumulative release of vancomycin between group B (110.2 mg/l) and group F (251.2 mg/l) ($p < 0.001$). It was concluded that in order to enhance antibiotic release from spacers, surgeons should manually incorporate high antibiotic concentrations into the most appropriate bone cement and keep the interim period as short as possible. Authors suggests that manual incorporation of 4 g of vancomycin to every 40 g of gentamicin premixed "Palacos R + G" to create bone cement spacers.

Goyal et al. [50] performed an in vitro elution release analysis of piperacillin and tazobactam from bone cement, in combination with gentamicin loading. The authors made five different formulations including a sample A without any antibiotic (control), a sample B with 4g piperacillin and 0.50g tazobactam, a sample C with 6g piperacillin and 0.75g tazobactam, a sample D with 8g piperacillin and 1g tazobactam and a sample E with 4g piperacillin and 0.50g tazobactam, and 400 mg gentamicin. Researchers observed detectable levels of elution for piperacillin and tazobactam for 21 days, with the highest levels occurring on day 2. Piperacillin release showed much sharp decline in drug levels as compared with tazobactam. About 0.8 -1.2% of piperacillin and 23–29% of tazobactam were released from the samples. The presence of gentamicin significantly improved elution from bone cement for both piperacillin and tazobactam in sample B and E ($p = 0.000$). In conclusion, piperacillin and tazobactam eluted successfully from bone cement and also retained antimicrobial activity after elution. Maximum elution was seen up to day 2 and antimicrobial action was seen up to 7 days.

Humez et al. [44] investigated the mechanical stability, handling properties, and elution behavior of PMMA cement loaded with three different daptomycin concentrations in comparison to commercially available antibiotic-loaded bone cement. Two reference formulations were used from commercially available PMMA cement, reference 1 PMMA +0.5g gentamycin and reference 2 PMMA 0.5g gentamycin + 2g vancomycin. In addition, three test formulations, test 1 PMMA+ 0.5g gentamycin+0.5g daptomycin, test 2 PMMA+ 0.5g gentamycin+1g daptomycin, and test 3 PMMA+ 0.5g gentamycin+1.5g daptomycin were made. The highest volume of gentamicin release was observed on day 1, followed by a continuous decrease in antibiotic release. Samples of higher daptomycin concentrations had greater gentamicin release during five days. Test 3 had the most gentamicin release when compared to test 1 and test 2, suggesting a synergistic elution effect. For test 3, daptomycin's total release was greater (1039μg) than gentamicin's (734μg). A twofold increase in the rate of antibiotic release was seen when 0.5g of daptomycin was added: test 3 released 1039μg, test 2 released 611μg, and test 1 released 264μg. When reference 2's vancomycin elution was evaluated, it was found to have the greatest initial release of all examined samples on day 1 (1460μg), which was drastically reduced to 221μg on day 2. The total amount of daptomycin released was less than that of vancomycin from reference 2, suggesting a better overall elution from the whole amount of daptomycin. References 1 and 2 displayed bending strengths of 71 MPa and 58 MPa, respectively. Test 2 had the highest ISO bending strength (72 MPa), followed by test 1 (70 MPa) and test 3 (67 MPa). The two reference samples' bending moduli, which were 2922 MPa and 2900 MPa, respectively, were similar, meeting the minimal requirement of 1800 MPa. With a bending modulus of 3342 MPa, test 2 was the highest, followed by test 3 (3148 MPa) and test 1 (3120 MPa). Reference 1's ISO compressive

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strength of 87 MPa was greater than Reference 2's 78 MPa, which met the minimal requirement. The compressive strength was the highest for test 3 (93 MPa), followed by test 1 (92MPa) and test 2 (90 MPa). All test cement samples fulfilled the requirements for mechanical stability according to DIN 53435. Reference 2 reached a DIN bending strength of 69 N/mm^2 and Reference 1 of 81 N/mm^2. The DIN bending strength decreased with the increase in the daptomycin concentration, from 74N/mm^2 (test 1) to 70 N/mm^2 (test 2) and 64 N/mm^2 (test 3). Regarding DIN impact resistance, reference 1 (3.5 kJ/m^2), test 2 (3.2 kJ/m^2), and test 1 (3.1 kJ/m^2) exceeded the impact resistance of reference 2 (3.0kJ/m^2, while GD1.5 (2.6 kJ/m^2) showed the highest difference in DIN impact resistance. The higher the daptomycin concentration, the lower the measurements for DIN bending strength and impact resistance, indicating that a high daptomycin concentration in PMMA bone cement reduces its mechanical properties. In conclusion, PMMA cement containing 0.5 g of gentamicin and 1.5 g of daptomycin could be a good alternative to the already established COPAL® (Wehrheim, Germany) G+V for the treatment of PJIs caused by vancomycin-resistant Enterococci.

In a recent study, **Pedroni et al.** [64] evaluated different concentrations of vancomycin and/or gentamicin-loaded PMMA again biofilm formation of Staphylococcus aureus. For this purpose, 8 groups of specimens were developed. In the first group (V1), 1g of vancomycin was loaded to PMMA and in the other two groups were included 2g (V2) and 4g (V4) of vancomycin, respectively. The authors also included three groups where a combination of 500 mg of gentamicin with vancomycin 1g (V1G), or 2g (V2G), or 4g (V4G) were used. Then, one group with gentamicin (500mg) alone (G) and another control group only with PMMA (C—control) were included. Different surface features were observed by SEM (20x) based on the concentration of antibiotics. V4 exhibited increased surface area, roughness, and severe porosity. When compared to raw PMMA and other groups (V1, V2, V1G) that displayed reduced concentration of vancomycin and gentamicin, while the group V4G displayed an exceptionally rough surface, which increased the likelihood of biofilm. The V2G and V4G had a similar surface. In conclusion, effects against adherence and bacterial development in PMMA loaded with antibiotics were mainly seen in the group vancomycin 4 g + gentamicin 500 mg, and a synergic effect can be applied in antibiotic-loaded cement.

Other Formations of PMMA

Ikeda et al. [26] created a double-layered antibiotic-loaded cement spacer in which calcium phosphate cement is coated with PMMA cement to enhance its mechanical properties. Double-layered PMMA spacers were loaded with vancomycin and immersed in phosphate buffer for 84 days. To facilitate vancomycin elution from calcium phosphate cores in double-layered spacers, authors drilled multiple holes into the calcium phosphate layer. For both double-layered and single PMMA spacer, vancomycin elution was high on day 1 and reduced gradually. However, vancomycin concentration in double-layered spacer eluents surpassed those in PMMA spacer eluents especially significantly on and after day 7 ($p = 0.016$ on day 7; $p = 0.0079$ on and after day 14 and until day 84). Because of the perpendicular load of the spacer surfaces, cracks entered the spacers perpendicularly and authors found that the compressive strength of the double-layered spacer (mean, 7.3 kN) was significantly lower than that of the PMMA spacer (mean, 15.1 kN) ($p = 0.0079$). However, this compressive strength value is much higher than the 5.64 kN, that corresponds to a load of 575 kg. It was concluded that the beneficial biomechanical and drug-eluting properties of the double-layered spacer might qualify it to serve as a promising biomaterial that could be used for managing periprosthetic joint infections.

Luo et al. [28] compared the antibiotic elution characteristics of a composite drug delivery system consisting of PMMA/calcium sulfate carrying vancomycin (dual carrier-v) to a PMMA loaded with vancomycin (PMMA-v) as a control. Vancomycin was progressively released from the dual carrier-v and the PMMA-v up to about 8 and 6 weeks, respectively. During the release time, the dual carrier-v presented higher levels of vancomycin release compared to PMMA-v ($p < 0.05$). In conclusion, the results suggest that the dual carrier-v can release higher concentrations of antibiotics and inhibit bacteria growth more effectively in vitro as compared with PMMA-v and thus the dual carrier-v may have potential as an alternative strategy for osteomyelitis management.

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Labmayr et al. [53] applied a method of superficial vancomycin coating (SVC) to strengthen the cements' antibiotic effect and its mechanical properties. Two commercially available PMMA bone cements were used, and four groups were formed. The authors noticed that the antibiotic effect was enhanced by the presence of SVC in both cements at every time point within 24 hours. The bending modulus and bending strength of Palacos with SVC (2089 MPa, 61 MPA) and Copal with SVC (2283MPa, 57 MPa) were significantly above ISO requirements. In conclusion, SVC boosts the antibiotic effect of ALBCs in the first 24hours, while maintaining sufficient stability.

In the current study of **Alimohammadi et al.** [56], authors tested the distribution of pores characteristics of bone cement specimens with different amounts of telavancin. The objective of this study was to provide statistical descriptions for the pore distribution characteristics of laboratory bone cement specimens with different amounts of antibiotic contents. Four specimen groups were created containing 0.3, 0.6, 1.2 and 2.4 wt./wt.% of telavancin. The porosity percentage, which was 2.0% for the 0.3 wt./wt.% group, increased gradually to 3.4% for the 0.6 wt./wt.% and to 4.4% for the 2.4 wt./wt.% groups ($p < 0.05$). The density of small micropores (diameter <0.1 mm) was consistently large in all groups, although the volume fraction of these pores was unimportant. For larger micropores (0.1–0.5 mm diameter), both the density and the volume fraction were considerable in all groups. The micropores of 0.5–1.0 mm had a limited frequency, with no substantial difference between the groups. It was found a significantly larger porosity in groups with larger added antibiotic contents, and it was suggested that micropore clusters have a detrimental effect on the mechanical properties of bone cement and play a major role in initiating fatigue cracks in highly antibiotic added specimens.

Porogens/Fillers

PMMA is non-porous so less than 5% of the loaded antibiotic is released. To enhance antibiotic elution and obtain better release of the antibiotics, greater porosity of the beads is required. Adding fillers and porogens could increase the bead's porosity, thus improving the antibiotic release from the beads.

Slane et al. [14] explored the usage of low concentrations of xylitol (0g, 1g, 2.5g, 5g, or 10 g) in a gentamicin-loaded cement as filler. In dry samples, flexural modulus increased linearly with increasing xylitol concentration and the addition of 10 g of xylitol increased the flexural modulus by 25%. Conversely, flexural strength decreased linearly with increased xylitol loading, with a 21% decrease observed with the addition of 10g xylitol. The addition of xylitol did not show any relationship with compressive modulus or yield strength. After immersion in PBS for 3 weeks, the flexural strength decreased by 23% post-submersion relative to dry PMMA. Both the flexural compressive properties decreased significantly with increasing xylitol concentration and demonstrated a power law relationship. For flexural properties, however, the yield strength for all wet samples except those with 10 g xylitol was higher than the ISO standard of 70 MPa. Analysis of the SEM micrographs revealed that the fracture surface of the four-point flexural samples tended to increase in roughness and tortuosity with higher xylitol content. Authors found that fracture toughness decreased linearly with increasing xylitol content. The addition of xylitol significantly ($p < 0.001$) increased the porosity of the cement. A strong positive linear correlation was found between xylitol content and porosity ($R2 = 0.997$). Relative to standard Palacos R+G, the addition of 10 g xylitol increased the porosity by 317%. The addition of xylitol was found to have a significant ($p <0.05$) effect on the cumulative gentamicin release over 45 days. Even with the addition of 1g of xylitol, there was a significant increase in gentamicin release compared to the standard cement. Interestingly, between the samples containing 2.5 or 5g of xylitol, there was a slight difference in gentamicin release, 33.4% and 34%, respectively. Authors concluded that xylitol-modified bone cement may not be appropriate for implant fixation but could be used in instances where sustained, increased antibiotic elution is warranted, such as in cement spacers or beads.

Rasyid and Soegijoko [15] investigated glycine (0.6g) and sodium chloride (0g, 12g, 16 g, 20g, and 24g) as potential fillers in PMMA. The addition of glycine yielded a 16% release of the total amount of gentamicin incorporated in 24 hours. Subsequent addition of sodium chloride resulted in

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an increased gentamicin release. In the mixture of gentamicin, glycine, and 24g sodium chloride, the researchers recorded the highest release of gentamicin, with the antibiotic reaching a peak level of 2222.561 μg/mL at 168 h. In conclusion, the addition of glycine and sodium chloride resulted in an increased release of gentamicin.

In the study of **Funk et al.** [25], authors evaluated the effect of adding a borate bioactive glass (13-93B3) on vancomycin elution from PMMA bone cement. Five cement groups were prepared, each with different compositions BG (0g vancomycin, 10g glass), V1 (1g vancomycin, 0g glass), V5 (5g vancomycin, 0g glass), BGV1 (1g vancomycin, 10g glass) and BGV2 (5g vancomycin, 10g glass). BGV1 showed significantly greater cumulative vancomycin elution over V1 ($p<0.001$) and this difference was spotted in vancomycin concentration every day until Day 10 ($p<0.05$). In specific, BGV1 released 87.56% more vancomycin by mass than V1 over 14 days. Similarly, BGV5 showed significantly greater cumulative elution of vancomycin compared to V5 ($p<0.001$). which was obvious for many days. BGV5 released 20.76% and 21.10% more vancomycin by mass than V5 over 14 and 28 days, respectively. Both groups presented their highest compressive strength at Day 0, which decreased over time. Although, the compressive strength of all groups didn't drop below 70 MPa throughout the experiment. Similarly, Young's modulus gradually fell over time for both groups. In conclusion, the incorporation of borate bioactive glass into commercial PMMA bone cement can significantly increase the elution of vancomycin with a mechanical strength of the cement-glass composites, suggesting their suitability for orthopedic weight-bearing applications.

A few years later, **Chen et al.** [13] investigated the optimum dosage of gelatin (10%, 20%, 30%, 40%, and 50%), as porogen. Indeed, the addition of gelatin resulted in a porous structure, which benefited the mechanical property of the PMMA bone cement and increased the gentamicin release. The constructs with lower porosity had reduced burst rate and relatively shorter release durations. Compared to the standard PMMA bone cement, the porous structure reduced the mechanical and thermal properties, in specific the component with 200–400 μm gelatin has better porosity, which resulted in the increasing drug release amount and rate. The compressive mechanical performance was affected by the increasing amount of the gelatin, especially after the immersion in SBF. Although the compressive strength descended sharply with the promotion of the gelatin from 10% to 50%, ranged from 67.6 MPa to 21 MPa, respectively and it still lied between the compressive strength of cancellous (10 MPa) and cortical bones (60 MPa). In conclusion, data analysis and fitting curve could guide experiments to obtain the PMMA bone cement with specific requirements of the mechanical properties by the addition of gelatin as the pore-forming agent, and also to estimate how a change of gelatin may affect the porosity, mechanical properties, and drug release profiles.

Recently, **Chen et al**. [21] also constructed a gentamicin sulfate (GS)/alendronate (ALN)-dual-loaded gelatin-modified PMMA bone cement (GAPBC) to provide rapid and continuous gentamicin release. GAPBC with 20% GS/ALN-dual-loaded gelatin microspheres exhibited a slightly higher gentamicin release profile than the other groups, reaching 73.6 ± 1.75% at the equilibrium state. Differences in the method of loading gentamicin into the gelatin microspheres resulted in the release profile from GAPBC being significantly greater than that from PMMA bone cement, although the initial gentamicin dosage loaded into the GAPBC was less than that in PMMA bone cement. However, the trend of the alendronate release was relatively different from that of the gentamicin release. In conclusion, GS)/ALN-dual-loaded gelatin modified PMMA bone cement (GAPBC) represents a potential drug carrier for future clinical applications.

Additives

It has been shown that most of the loaded antibiotic remains embedded in PMMA polymer and does not participate in drug elution. Incorporation of the various additives to ALBC can help to overcome these issues.

To enhance the antibiotic release properties of PMMA cements, **Qin et al.** [9] assessed the addition of strontium-doped calcium phosphate spheres (SCPS), using unmodified PMMA as the control and PMMA+2.5% wt. vancomycin, PMMA +10% wt. SCPS (including 10% wt. vancomycin) and PMMA+2.5% wt. vancomycin+10% wt. The cumulative release of vancomycin revealed a burst

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release that followed by a sustained release in all groups. Vancomycin release from PMMA+2.5% wt. vancomycin+10% wt was the most rapid, followed by the elution from PMMA+2.5% wt. vancomycin and PMMA +10% wt. SCPS (including 10% wt. vancomycin). The incorporation of SCPS in PMMA cement hence facilitated drug release. Authors didn't notice significant differences in either compressive strength ($p = 0.085$) or elastic modulus ($p = 0.446$) between control PMMA and PMMA +10% wt. SCPS (including 10% wt. vancomycin), suggesting that the SCPS incorporation did not affect PMMA's mechanical properties. The results showed that SCPS/PMMA antibiotic-loaded cement had enhanced antibiotic release, delivered strontium ions and maintained mechanical properties, indicating that the SCPS additive could be a suitable alternative for controlling the drug-delivery properties of PMMA cement.

Oh et al. [29] examined the Pluronic F68 (EG79PG28EG79) as a hydrophilic additive to PMMA cement and its effect on vancomycin elution. Several PMMA samples were constructed with different concentrations of Pluronic F68 (0%, 1%, 3%, 5%, 7%, and 10% wt.) and 2g of vancomycin. The control cement without Pluronic F68, revealed a limited release of vancomycin for the first two weeks (15%), and thereafter the release of antibiotic from the bone cement was not significant. With the Pluronic F68+vancomycin cements, the release period and percentage of the antibiotic raised with increasing concentrations of Pluronic F68 up to11 weeks, supporting that the Pluronic F68 can improve vancomycin release from the PMMA bone cement. However, the compressive strength was decreasing as the concentration of Pluronic F68 increased, possibly caused by the plasticizing effect of the Pluronic F6838 and/or reverse micelle formation in the PMMA bone cement. It was also recognized that the compressive yield stresses of the most of Pluronic F68+vancomycin cements fulfill the ISO criteria for clinical application. Authors suggested that the use of Pluronic F68 as a hydrophilic additive for antibiotic-eluting PMMA bone cement can be a promising strategy to treat osteomyelitis.

Zhou et al. [41] assessed the release of vancomycin and tobramycin from CPP (calcium polyphosphate) gel-impregnated PMMA cement (Simplex P, SP) and the influence of the impregnation of CPP hydrogel on the mechanical strength of bone cement. Two PMMA groups were produced, a PMMA +antibiotics (control group) and a CPP-PMMA +antibiotics group (CPP group). The researchers analyzed the release of antibiotics from both groups for up to 24 weeks. During the first 72 hours, the CPP group showed a significant reduction in burst release of vancomycin compared to the control group ($p<0.05$), and a similar pattern of tobramycin release was also observed. Moreover, the CPP group exhibited an extended release of both vancomycin and tobramycin, starting from 1 week and continuing up to 24 weeks. The release rate of combined antibiotics showed a statistically significant difference from week 7 until week 24 ($p<0.05$). Reconstructed three-dimensional morphology revealed no significant difference between groups. After immersing in PBS for 24 weeks, both groups become less dense and show significant surface defects, especially around the edges. CPP group presented slightly higher porosity and pore size than the control group prior to and after antibiotic release. The contact angle between groups was equivalent, while the surface roughness was considerably improved in the CPP group. CPP PMMA consisted of evenly and more densely packed nanoprojections. The surface roughness of the CPP group was significantly higher for SPC1VT. After soaking in PBS for 24 weeks, noticeable circular defects were found on the surface of the CPP group, which were not evident on the surface of the control group. CPP group revealed a minor reduction in the ultimate stress (122 MPa) as compared to the control (128 MPa). The elastic modulus of the CPP group (2.5 GPa) was found to be higher than the control (2.09 GPa). The authors concluded that this new material is biocompatible and has similar handling properties and mechanical strength as compared to SP cements. We believe that incorporating CPP gel provides a better and usable drug carrier for PMMA cement.

In a study by **Cyphert et al.** [60], insoluble cyclodextrin (CD) microparticles are incorporated into PMMA to provide more sustained delivery of antibiotics without affecting the mechanical properties of the PMMA formulations. For this purpose, several groups of cylindrical samples were prepared, including plain PMMA cement and cement with tobramycin only, rifampicin only, gentamicin only, empty 5 wt.% β-CD microparticles, empty 10 wt.% β-CD microparticles, 5 wt.%

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tobramycin-β-CD microparticles, 10 wt.% tobramycin-β-CD microparticles, 5 wt.% gentamicin-β-CD microparticles, 10 wt.% gentamicin-β-CD microparticles, 5 wt.% rifampicin-β-CD microparticles, and 10 wt.% rifampicin-β-CD microparticles. Gentamicin and tobramycin release plots demonstrated a relatively decreasing linear trend over the 16 days for all PMMA formulations. The release of RMP was not reported because of its high hydrophobicity and affinity for CD. To evaluate the mechanical properties, seven different cement groups (plain PMMA, PMMA with tobramycin, PMMA with rifampicin, PMMA with 5 or 10 wt.% empty β-CD microparticles, and PMMA with 5 or 10 wt.% rifampicin-β-CD microparticles) were selected. PMMA with tobramycin was used as the control group since all the experimental groups have antibiotics incorporated into them. Adding either 5 or 10 wt.% β-CD microparticles resulted in a significant decrease in the compressive strength of PMMA (5 wt%: 70 MPa; 10 wt.%: 67 MPa) compared to free tobramycin ($p = 3 \times 10^{-7}$ and $p = 9\times 10^{-10}$, respectively). The addition of either 5 or 10 wt.% rifampicin-β-CD microparticles resulted in a similar compressive strength (5 wt%: 76 MPa; 10 wt.%: 76 MPa) to free. On the other hand, adding rifampicin led to an important decline in compressive strength (2 MPa) relative to free tobramycin (77 MPa). These findings suggest that addition of insoluble cyclodextrin microparticles to cement promotes post implantation antibiotic refilling and enables incorporation of previously incompatible antibiotics while preserving favorable mechanical properties.

Kilinc et al. [47] measured and compared the elution characteristics of teicoplanin from PMMA beads (group 1) with those of poly(glycolide-co-lactide) PGLA-added beads (group 2). Between two groups, teicoplanin concentration was statistically significantly different, being higher in Group 2 ($p < 0.001$). Authors suggested that the in vitro results showed that the addition of biodegradable PGLA into bone cement functions as a water-soluble porogen which allows for significant increases in the elution of teicoplanin from cement.

Tseng et al. [32] examined the effect of Vicryl Rapide sutures on the elution profile of vancomycin from PMMA beads and their mechanical properties. The selected Vicryl Rapide sutures are composed of a copolymer made from 90% glycolide and 10% L-lactide and are characterized by its fast absorption with total hydrolysis at 42 days. There was one control group (no suture) and three experimental groups: a passing suture group, a passing suture + segment group, and a segment group. The post-hoc analysis revealed that the "passing suture group" and the "passing suture + segment group" released more vancomycin than the "segment group" and the control group ($p < 0.001$). A burst release was observed in each group on day 1. Compared with the control group, the total release amount from the "passing suture group" increased by 39.9%. Regarding gross appearance, analysis of the SEM images showed that the sutures were almost completely hydrolyzed in the outer half of the beads (peripheral area) and antibiotic particles around the suture tunnel were absent, and their nest holes were left empty. In contrast to the round pores in the PMMA cement, their shape was irregular and consistent with that of the antibiotic particles. In the control group, numerous antibiotic particles were still densely entrapped in the outer half and center of the cement beads. In conclusion, passing fast absorbable sutures through PMMA cement is a feasible method to fabricate sustained-release antibiotic bone cement, the effect can last for at least 7 weeks, and it is suitable for a temporary spacer between two stages of a revision surgery.

Cherednichenko et al. [58] tested four different natural micro/nanoscale materials -halloysite HNTs, nanocrystalline cellulose (NCC), microfibrillated cellulose (MFC), and nanofibrillated cellulose (NFC)- as additives to PMMA bone cement preloaded with vancomycin. Composite ALBC (c-ALBC) demonstrated a higher vancomycin release compared to ALBC except for the NCC-containing PMMA. Among c-ALBC, the vancomycin release decreases in the row MFC > NFC > HNT > NCC. The vancomycin elution showed a phase of exponential increase during the first ten hours, which was followed by the declining phase and plateau in a few days. A significant increase in c-ALBC microhardness was observed because of the usage of additives. The PMMA samples containing NCC presented the highest microhardness value. Low magnification SEM micrographs revealed that the number of pores in the PMMA matrix increased significantly when the antibiotic and additives were introduced into its composition. In conclusion, authors observed the best

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combination of polymerization rate, monomer leaching, antibiotic release, and microhardness in the sample that contained nanofibrillated cellulose.

Nanocomposites

Letchmanan et al. [20] investigated the impact of mesoporous silica nanoparticles (MSNs) loaded with gentamicin (GTMC), vancomycin (VCMC) and tobramycin (TBMC) on the mechanical properties of PMMA. Seven different nanomaterial-formulated antibiotic bone cements were made, BC-1 (GTMC/MSN/Simplex-P- 2.72 wt.%), BC-2 (GTMC/MSN/Smartset-HV), BC-3 (GTMC/Xylitol/Simplex-P), BC-4 (GTMC/Simplex-P), BC-5 (GTMC/MSN/Simplex-P- 8.15 wt.%), BC-6 (GTMC/VCMC/MSN/Simplex-P) and BC-7 (GTMC/TBMC/MSN/Simplex-P). The incorporation of MSNs did not show a significant effect on the biomechanical strength as compared with the standard bone cements ($p < 0.05$). The BC-1 and BC-2 preserve at least 80% of the original bone cement strength, showing both compressive strength of more than 75 MPa after 6 months of aging and the bending modulus above 5GPa. Satisfactory mechanical properties observed for BC-5 with 8.15 wt.% of MSNs, even after being aged for 6 months. In the meantime, BC-3 and BC-4 have shown a reduction of more than 20% in both the compressive strength and bending modulus within the first month relative to original Simplex-P. The compressive strength of the bone cements fell below 70 MPa after one month. Despite observing some decrease in mechanical properties compared to the single-drug systems, all the formulated cements exhibit mechanical strength higher than that of the ASTM F541 and ISO 5833 standards. No significant changes in the PMMA-based bone cement structures (pores and voids) could be observed because of the incorporation of MSNs. More than 10% of gentamicin release from MSN–bone cements (BC-1 and BC-2) on the first day, was observed and reached more than 55% of release over 77 days. On the other hand, the BC-3 bone cement and the high loading of GTMC without fillers (BC-4) also did not show significant improvement in gentamicin release, although higher drug release profile than controls. Only about 10.5% and 16.8% of GTMC released have been observed for BC-3 and BC-4, respectively, for 77 days. In conclusion, the combination of excellent mechanical properties and sustainable drug delivery efficiency demonstrates the potential applicability of mesoporous silica nanoparticle-functionalized PMMA bone cements for orthopedic surgery to prevent post-surgery infection.

Shen et al. [10] examined the effect of hollow nanostructured titanium-dioxide (TiO_2) nanotubes (TNTs) on drug release profiles for antibiotics and mechanical properties (compression and bending) of bone cements. Antibiotics, such as gentamicin (GTMC) or vancomycin (VCMC), were loaded into PMMA based bone cement powder together with TNTs powder. Initially, plain bone cement exhibited low total drug release as only 5% of drug release was achieved on the first day, followed by tiny drug release throughout the rest of the test duration. With the presence of 6.3 wt.% TNTs in the bone cement formulation, the drug (gentamicin and vancomycin) release was obviously enhanced. The drug release was further enhanced with increasing TNTs loadings. Increasing the content of TNTs up to 18.5 wt.% the release of gentamicin was significantly increased and the accumulative release reached more than 40% in 70 days. When TNTs content was increased to 25.2 wt.%, more than 50% of loaded gentamicin was released in 70 days and the accumulative release reached about 50% in first two weeks, contributing to the major portion of total drug release. The following release kinetic after two weeks was much slower. At an increase of the TNTs content to 18.9 wt.%, the accumulative release of vancomycin reached 41.6% in 70 days. With 25.2 wt.% of TNTs, the total drug release reached 55%. It was found that when the content of TNTs was less than 25 wt.%, almost more than 95% of compression strength was preserved. As the content of TNTs increased to 31%, the compression strength of bone cement declined to 70 MPa, below the minimum compression strength requirement according to ASTM F541 and ISO 5833. It was also found that compression strength of TNTs formulated bone cement was not adversely affected after aging in BPS and some samples became stronger than the corresponding fresh as prepared samples. Although the loaded antibiotics in nanotube channels was released, the TNTs were not soluble in aqueous solution, thus the TNTs remained in the bone cement matrix to maintain the compression strength. Authors noticed that the formulation of TNTs has a small impact on their bending modulus. In bone cement, over 90%

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of the bending modulus was retained when the TNT level reached up to 25% weight percent. After three months of immersing in PBS, the bending modulus showed negligible changes, in line with the compression strength. An increase in TNT content in the formulation leads to rougher fracture surfaces and microporous structures, which accelerate drug elution and increase the overall percentage of drug release. After incorporating TNTs, the study showed that the mechanical properties of PMMA-based bone cements could be well preserved even after the drug release for 70 days or aging in PBS buffer for 3 months. Furthermore, more than 50% of loaded antibiotic (such as gentamicin or vancomycin) could be released in two months.

Perni et al. [16] developed silica nanocarriers loaded with gentamicin as a drug delivery system to be dispersed in poly methyl-methacrylate (PMMA) bone cement for controlling and extending the release of the antibiotic from bone cements, thus proving a prolonged antimicrobial activity. They used a layer-by-layer self-assembly to put gentamicin between alginate layers and two different poly β-amino esters (piperazine for POLY1 and 4,4′-trimethylenedipiperidine for POLY2) on their silica nanoparticles. Gentamicin release from both PMMA-coated nanoparticles was the highest at the beginning and continued to decrease for up to 25 days and the overall release from PMMA using POLY1 was the higher released. When POLY2 was employed as a polyelectrolyte, the gentamicin release was greater during the first 2–3 days than POLY1. Gentamicin release was almost finished after about 5–6 days when gentamicin was simply mixed in the PMMA dough. It was found that release of gentamicin from PMMA bone cement containing silica nanocarriers continued for about 30 days compared to 6 days when the same amount of antibiotic was added as a pure powder (as in commercial formulations). An extended antimicrobial property of the drug released from the carrier was also found while the mechanical properties of the bone cement were unaffected when the silica nanocarriers were added.

A couple of years ago, **Al Thaler et al.** [23] investigated the application of carbon nanotubes (CNTs) incorporated in PMMA on gentamicin release. PMMA with 3% gentamicin powder, PMMA with 3% gentamicin-loaded CNTs, PMMA with 0.3% CNT and 3% gentamicin powder, and PMMA with 1% CNT and 3% gentamicin powder types were produced and analyzed. Gentamicin-loaded CNT released more than 80% of gentamicin in the first 5 days. After day 5, gentamicin release reached a plateau, where no significant increase in gentamicin release was observed over time. All the CNT-containing bone cements released a significantly higher amount of gentamicin than the gentamicin powder-containing cement ($p < 0.05$). The gentamicin-loaded CNT cement demonstrated significantly higher percentage release (45%) than other types of bone cement ($p < 0.05$), which presented similar lower percentages (15%). However, the release continued for 25 days from all bone cement types tested at different concentrations and percentage levels according to the cement type. Also, the CNT-containing cements had similar compressive strength compared to the gentamicin powder-containing bone cement ($p > 0.05$). In conclusion, the results showed prolonged release of gentamicin from carbon nanotube-loaded bone cements over several weeks compared to gentamicin-containing bone cement with no compromise of mechanical characteristics of the bone cement.

Kehribar et al. [46] studied the biomechanical properties of PMMA structures with varying amounts of silver nanoparticles (AgNPs). Seven different samples were prepared consisting of a ratio of teicoplanin/cement 40/400 mg according to the supplier's procedure combined with 1,25mg, 2.5mg, 5mg, 7,5mg and 10mg of silver nanoparticles. As the amount of AgNP increased, this led to a shift to lower absorbed energy that revealed the increase in the structural fragility of the PMMA. The AgNP-loaded samples in various concentrations showed a similar maximum force durability. In conclusion, silver nanoparticles in various combinations enhance antimicrobial activity synergistically while maintaining the mechanical strength of bone cement.

Most recently, **Tavakoli et al.**[31] applied graphene oxide encapsulated baghdadite (GOBgh) nanoparticles as a radiopacifying and bioactive agent in PMMA bone cement containing 2 wt.% of vancomycin. Several PMMA bone cement samples were made by adding 0%, 10%, 20%, 30%, or 40% wt. of Bgh or GOBgh nanoparticles to PMMA powder. On the first day, vancomycin elution from all samples was high and its intensity decreased in the following days. Vancomycin eluted from the PMMA sample after 14 days was measured as 15.71 ± 1.37%, although this value remained constant

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until day 28. However, vancomycin elution for PMMA-Bgh20 and PMMA-GOBgh20 samples continued until day 21., where 27% and 29% of the incorporated amounts were released in 21 days, respectively. Based on the promising results obtained, the authors concluded that PMMA-GOBgh20 bone cement is suggested as an optimal sample for use in orthopedic surgeries.

Mixing Technique, Ultrasounds and Sonication

Martínez-Moreno et al. [40] examined ciprofloxacin release from three trademarks of bone cements and its bioactivity using as variables the mixing method (hand or vacuum), the chemical form of the antibiotic (base or hydrochloride) and the antibiotic combination by adding vancomycin. For this purpose, ten different formulations were made in total. When ciprofloxacin incorporated as a base released 35% of the amount released when she used in its hydrochloride form. Furthermore, more ciprofloxacin was produced when vancomycin and ciprofloxacin were combined. All samples of ciprofloxacin hydrochloride produced high early release rates, followed by a lower sustained release. In vacuum-prepared samples, significant differences were obtained in the total amount of antibiotic released between the two bone cement types (Simplex P and Lima CMT). Authors observed statistical differences between mixing procedures for one bone cement (Lima CMT). Palacos and Lima CMT have higher levels of porosity than Simplex, according to scanning electron microscopy. In conclusion, mixing techniques, the chemical form and the combination of antibiotics influence the antibiotic release and porosity if bone cements.

Pithankuakul et al. [30] studied the impact of different mixing speeds and techniques on the elution and mechanical properties of high-dose vancomycin-loaded bone cement that was prepared by hand. In total, 6 groups of PMMA samples were created, normal vs high speed mixing groups, which were then divided into three methods of antibiotics mixing (standard, 1-minute delayed and 5-minute delayed). The total cumulative vancomycin elution of the high-speed group was 24% for the 15 days of the experiment, much increased than that of the normal-speed group ($p < 0.001$). It was observed that the vancomycin elution rates were significantly higher in the delayed antibiotic addition groups (0.178 mg/ml for the 1-minute delayed mixing group and 0.181 mg/ml for the 5-minute delayed mixing group) compared to the standard direct antibiotic mixing group (0.156 mg/ml) ($p < 0.05$). The overall compression strength of all preparations was significantly decreased and after 15 days of elution compared to that of before the initiation of elution (64.47 MPa and 72.65 MPa, respectively) ($p < 0.001$). Authors noticed that the compression strength of the high-speed group was lower than that of the normal-speed group ($p< 0.001$). It was also observed that the high-speed group had a greater percent of porosity than the normal-speed group ($p< 0.001$). Additionally, researchers observed a relationship between higher percentages of total porosity and higher vancomycin elution levels. In conclusion, bone cement prepared with high-speed hand mixing and delayed antibiotic addition can exhibit increased vancomycin release and a greater percent of porosity.

Wendling et al. [33] investigated whether combining modifications in the mixing technique of antibiotic-impregnated PMMA cement with low-frequency ultrasound (LFUS) improves vancomycin elution and affects mechanical strength. Three groups of PMMA were prepared, each using a different preparation method, standard PMMA (vancomycin was added to the dry polymer and briefly mixed before adding the liquid monomer and then mixing for 90 seconds), “delayed” PMMA (the dry polymer and monomer were mixed for 60 seconds before the addition of vancomycin, followed by 30 seconds of mixing) and control PMMA (prepared using the standard technique without the addition of vancomycin). In comparing the two mixing techniques, the delayed technique revealed a significant increase in elution only at day 1 compared with the standard technique ($p < 0.001$). The conversion point from the initial high phase to the subsequent low phase took place on day 10 when the ultrasound treatments were initiated (days 10, 12, and 14). Vancomycin elution from all LFUS groups was significantly higher compared to the non-LFUS groups (all $p < 0.001$). Also, the delayed PMMA revealed notably higher elution volumes at the 5 min ($p = 0.004$) and 45 min ($p < 0.001$) duration of LFUS groups compared to standard PMMA. Regarding their mechanical properties, both standard and delayed PMMA exhibited a significant decrease in offset yield stress

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compared with the control PMMA ($p < 0.001$), nevertheless above the 70 MPa limit. The offset yield stress didn't differ significantly between the LFUS durations for both mixing techniques (p-value > 0.3). The delayed mixing technique resulted in a significant reduction in stiffness across all LFUS durations compared with the respective control groups (all $p < 0.03$). In conclusio**n**, the combination of a delayed mix technique with LFUS treatments provides a reasonable means for increasing both short- and long-term antibiotic elution without affecting mechanical strength.

Some years later, **Wendling et al.** [34] examined the effect of ultrasound frequency and treatment duration on vancomycin-impregnated PMMA antibiotic elution rates and mechanical strength. Two groups of PMMA were prepared, one containing 5g of vancomycin and one without that were divided into two frequency groups (kHz and MHz) and two treatment duration groups (2 minutes and 10 minutes). All ultrasound treatment groups produced substantially increased vancomycin elution compared to the non-ultrasound group on day 1 of treatment. Both ultrasound frequency and treatment duration had significant univariate effects on increasing antibiotic elution ($p < 0.001$). Both kHz frequency groups produced significantly greater antibiotic elution than the MHz groups ($p < 0.001$). The 10-minute duration groups produced significantly greater antibiotic elution than the 2-minute duration groups (both $p < 0.001$). For offset yield stress, both ultrasound frequency and treatment duration showed no significant univariate effects ($p = 0.841$ and $p = 0.179$, respectively). For elastic modulus, US frequency had a significant univariate effect ($p = 0.024$), but treatment duration did not ($p = 0.136$). All groups maintained offset yield stresses significantly above the 70 MPa limit specified by ISO 5833:2002 ($p < 0.001$). In conclusion, ultrasound frequency and treatment duration significantly affect antibiotic elution from PMMA, which may be helpful for treatment of periprosthetic joint infections during revision arthroplasty.

Kummer et al. [51] hypothesized that the sonication may improve elution of antibiotics from PMMA. Thus, the authors evaluated in vitro the effect of sonication on antibiotics' elution properties from PMMA samples. PMMA blocks impregnated with vancomycin, fosfomycin, gentamicin or daptomycin were incubated in phosphate-buffered saline (PBS) for up to 6 weeks. Fosfomycin, daptomycin, and gentamicin showed a burst release during the first 7 days, with mean concentrations of 414.3µg/ml, 31.4µg/ml, and 83.2µg/ml, respectively. Vancomycin release remained stable over the 6 weeks (mean concentration 6.6–8.8 µg/ml) and above the MIC for most bacteria. Concentration of fosfomycin fell to 13.3 µg/ml after 2 weeks of incubation and became undetectable after 6 weeks and concentration of daptomycin declined gradually from 5.9 µg/ml after 2 weeks to 2.6 µg/ml after 6 weeks. Gentamicin concentration decreased from 17.8 µg/ml after 2 weeks to 6.0 µg/ml after 6 weeks. With sonication, the elution of antibiotics tends to increase. Specifically, authors detected a significant increase in vancomycin concentrations at 2 weeks ($p=0.008$) and 4 weeks ($p=0.002$), and in fosfomycin concentrations at 2 weeks ($p=0.01$). The effect of sonication could play a role in clinical results, especially for daptomycin and gentamicin, for which the MIC is close to the concentration of antibiotics at 4 and 6 weeks. In conclusion, the effect of sonication could play a role in clinical results, especially for daptomycin and gentamicin, for which the MIC is close to the concentration of antibiotics at 4 and 6 weeks.

Trabecular metal implants with an internal cemented interface may be customizable drug delivery devices with an ingrowth interface. **Mooney et al.** [17] analyzed thirty-six acetabular implants in vitro, half with a trabecular metal shell and half without. The antibiotic-loaded PMMA was prepared via three different mixing techniques (one hand-based and two mechanical-based on the Hivac 7 cementation system where mixing was performed either under atmospheric pressure or using a vacuum) and by applying two different mixing times (a short one consisted of mixing for 30s and doughing for 90s, and a long one of mixing for 90s and doughing for 30s). Mixing time has a significant effect on the total amount of gentamicin eluted with and without trabecular metal shells at 4 h and 7 days (Figure 2). The most significant and consistent effect was seen on the Hivac 7 with vacuum mixing protocol, where a longer mixing time improved the elution of all samples at all time points by as much as 226% ($p<0.001$) of that eluted by the short mix group. The total amount of gentamicin eluted showed that trabecular metal had a limited effect. Among the three cement mixing protocols, the long hand-based mixing protocol demonstrated the greatest porosity, while the short

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mechanical-based vacuum mixing protocol demonstrated the most limited pore structure. In conclusion, the establishment of trabecular metal as an effective delivery vehicle for antibiotics makes possible an entirely new class of drug eluting device designs.

Tranexamic acid (TXA) is an antifibrinolytic agent that is currently applied intravenously or locally into the joint space during joint arthroplasties and has significantly changed blood management by minimizing the need for transfusions [65]. **Lüdemann et al.** [18] assessed the effect of TXA on elution properties and the compressive strength of gentamicin-loaded PMMA. The authors noticed that at each of the 7 daytime points, there was no significant difference in the concentration of gentamicin released ($p_{1,3,5} = 0.055$, $p_2 = 0.423$, $p_{4,6} = 0.200$, $p7 = 0.522$). After immersion for 7 days, the cumulative concentration of gentamicin was also not significantly higher than that with the presence of TXA in the solution ($p = 0.297$). In terms of compressive strength, after 7 days of immersion, there was no difference in between the two study groups ($p = 0.262$). In conclusion, for the PALACOS R + G specimens, the addition of TXA did not produce significant decreases in gentamicin concentration, in the activity of the gentamicin eluate against a clinical isolate of *S. aureus*, the zone of inhibition of *S. aureus*, and in the compressive strength of the cement, after 7 d of immersion in the test solution.

Conclusion

In vitro studies on PMMA bone cement have provided valuable insights into the dual functionality of this material as both a structural support and a drug delivery system. The antibiotic elution profiles are highly affected by various variables, including the type and concentration of antibiotics, cement composition, and preparation methods. Simultaneously, these modifications can affect the mechanical characteristics, posing a challenge to maintaining the essential equilibrium between effective antibiotic release and structural integrity. Even though our understanding of these dynamics has been advanced significantly, further research is still required to optimize PMMA bone cement compositions. Future studies should focus on exploring novel additives, porogens and fillers, alternative antibiotics, and advanced manufacturing techniques in order to enhance both the therapeutic efficacy and mechanical performance. Addressing these challenges will be crucial for advancing the clinical applications of PMMA bone cement, ultimately improving patient outcomes in orthopedic and trauma surgeries.

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