Research Article

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Modification of methacrylate bone cement with eugenol – A new material with antibacterial properties

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Abstract: Nowadays, the search for unconventional antibacterial agents is very common. One of them may be eugenol (EU) (4-allyl-2-methoxyphenol), which exhibits antimicrobial properties against pathogenic bacteria and is used in the pharmaceutical industry. Owing to its structure, EU decreases the exotherm of polymerization without a negative impact on the degree of conversion. The properties of EU-modified bone cement, such as doughing time, maximum temperature, and setting time, will be characterized, as well as mechanical properties, EU release, and antibacterial properties. Bone cements were synthesized by mixing a powder phase composed of two commercially available methacrylate copolymers (Evonic) and a liquid phase containing 2-hydroxyethyl methacrylate, methyl methacrylate, triethylene glycol dimethacrylate, and EU with an amount of 0.5 wt% of bone cement sample. As an initiating system, benzoyl peroxide and *N*,*N*-dimethylaniline were used. Samples were prepared with various amounts of the initiating system. The doughing time, maximum temperature (T_{max}), setting temperature (T_{set}), setting time (t_{set}), and compressive strength tests were determined according to the ISO 5833:2002 standard requirements. The doughing time for bone cement depends on the amount of the initiating system. The maximum temperature during curing of bone cement is very low; however, the setting time is closer to the upper limit set by the standard. The compressive strength of the tested materials is good and significantly exceeds the requirements of the standard. EU release was very high and ranged from around 43–62% after 168 h. Moreover, antibacterial studies show that the tested bone cements are bacteriostatic for *Staphylococcus aureus* or and *Escherichia coil* strains. In summary, modified bone cements meet the ISO 5833:2002 standard requirements in all parameters and are characterized by good mechanical properties (similar to or higher than commercial bone cement), high EU release, and bacteriostatic properties.

Keywords: bone cement, antibacterial agents, eugenol, drug release, antibacterial properties

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1 Introduction

Bone cements are biomaterials designed to stabilize complex fractures as well as fix implants. There are a few types of commercially available bone cements, such as acrylic, calcium phosphate, and glass polyalkenoate (ionomer), but various types of other bone cements are still being developed [1]. Methacrylic cements are widely used in orthopedics and are based on poly(methyl methacrylate) (PMMA). These cements are two-component systems obtained in the

polymerization reaction by mixing the powder phase (solid polymer) with the liquid phase (mixture of monomers) [2]. The advantages of these cements are short curing time, simple preparation and application, good mechanical strength, and biostability in the human body. However, these materials require improvement of some parameters, such as the curing temperature, adhesion to bone and metal and appropriate contrast and antibacterial properties [3].

Antibacterial properties are required due to the introduction of a foreign object into the patient's body during the operation, the entry portal for microorganisms. Materials such as endoprosthesis or bone cement carry the risk of infection, which can be caused by direct contamination of the biomaterial or surrounding tissue, as well as blood and bone infection or the spread of surface infection [4]. In the case of endoprosthesis insertion surgery, the spectrum of bacteria leading to postoperative infections is broad and includes coagulase-negative staphylococci, streptococci, enterococci, Gram-negative bacilli, anaerobes, multiple pathogens, or unknown microbes [2,5]. The antibacterial activity of bone cements is achieved primarily by adding antibiotics [1,6–11], but also different antibacterial agents, such as silver [2,12,13], gold [2,14], or copper nanoparticles [15], as well as hydroxyapatite [16,17], bioactive glass [13,18,19], graphene oxide nanosheets [20,21], essential oils [2,22,23], for example, peppermint oil [2,24], or various types of methacrylic derivatives of active substances, such as methacrylate derived from benzothiazole (BTTMA) [25], quaternized ethylene glycol dimethacrylate piperazine octyl ammonium iodide (QAMA) [26], and others. Over the years, gentamicin has been the most commonly used antibiotic in bone cements since it provides treatment for various bacterial infections [2,10]. Among others, antibiotics loaded into bone cement are vancomycin, used in the treatment of Gram-positive-caused infections, and tobramycin, used in the treatment of Gram-negative-caused infections [10,27]. Emerging infections have become very complex to treat, caused by the wide range of microorganisms and the large number of antibiotic-resistant strains that lead to infections. Moreover, the formation of a bacterial biofilm on the surface of materials or the presence of multibacterial infections significantly complicates treatments [5,8]. This requires the use of nonclassical antibiotics or a combination of antibiotics added to bone cement [28–32].

The addition of antibiotics in powder form into bone cements to obtain antibacterial properties affects the material properties, such as mechanical strength. According to the literature [7,10,33], properties such as compressive and tensile strength and Young's modulus are not significantly affected, but it depends on the amount and type of incorporated antibiotic. Kühn [34] studied the compressive strength of several antibiotic-loaded bone cements and showed a decrease in the compressive strength value with the increasing amount of antibiotics compared to non-loaded bone cements. In turn, Boelch *et al.* [7] studied the compressive strength of two commercial bone cements: Copal® spacem and Palacos® R + G, at different vancomycin loadings in the powder phase of the cements. The results obtained showed that the addition of the antibiotic to the bone cement resulted in a slight decrease in the compressive strength irrespective of the amount of the antibiotic; however, after rinsing out the antibiotic, a significant decrease in compressive strength was observed [35].

A crucial parameter of the antibacterial performance of antibiotic-loaded bone cement (ALBC) is the release of antibiotics. It occurs mainly by volumetric diffusion. However, the rate and amount of release depend on the roughness of the surface, porosity, wettability of the material, and the distribution of the antibiotic in the polymer matrix [7,10,36]. It is very likely that a combination of diffusion and release from surface and volume defects, such as fractures and voids, contributes to the elution process. According to the literature, the rate of antibiotic release from antibiotic-loaded bone cement is low and ranges from 4 to 17% in various studies [6,37–39]. The release profile also affects the type of antibiotic. Studies have shown that the elution of PMMA is unique to particular antibacterial agents [7,10]. Antibiotics are released from bone cements either continuously or intermittently. For example, gentamicin has a continuous release kinetics, while vancomycin's release is characterized by a high initial release followed by a steep decline [7,40]. Gálvez-López *et al.* [41], in their studies, showed that antibiotics have individual release behavior. To increase the release of antibiotics from bone cements, they can be incorporated in the liquid form. It has been shown that liquid gentamicin is more effectively released from bone cements and retains its antibacterial effect [42,43], and liquid gentamicin combined with vancomycin in the same cement sample showed a positive mutual effect on the release of both antibiotics [43]. The improved elution of antibiotics in liquid form from bone cement is due to the increase in porosity of the material. In addition, the introduction of the antibiotic in a liquid form into the bone cement may provide a more efficient release than loading it with the same dose in the powder form [43,44]. However, the addition of antibiotics in the liquid form to the bone cement can reduce the mechanical properties of the material by up to 50% [45]. In studies reported by Hsieh *et al.* [43], the compressive strength of Simplex® bone cement with liquid gentamicin was reduced by 37%.

The inconvenience described above, such as the high bacterial resistance to conventional antibiotics or their ineffective release, prompted the search for other unconventional antibacterial agents. One of the interesting approaches is the use of different types of nanoparticles. However, nanoparticles have limitations, and there is a need to improve their antibacterial activity against certain bacterial strains, such as *E. coil.* In comparison, essential oils are safer and more reliable than synthetic drugs, which may have side effects [2,46]. One of the promising essential oils may be the naturally occurring eugenol (EU) (4-allyl-2-methoxyphenol), which is an aromatic hydroxyphenylpropene extracted from clove oil [47,48]. It is used in the pharmaceutical industry as an analgesic, anti-inflammatory, antiviral, antifungal, antiseptic, antispasmodic, antiemetic, and topical anesthetic. It exhibits antimicrobial properties against pathogenic bacteria [48] of both Gram-negative and -positive bacteria and antioxidant properties [6,47]. Its antibacterial activity is attributed to the presence of a free –OH group in the structure [49]. The minimum inhibitory concentration (MIC) of EU for *Helicobacter pylori* (both sensitive and resistant strains) and *Escherichia coil* (NCIM-2089) is 2 and 1 $\mu g \cdot ml^{-1}$, respectively [48].

The use of essential oils as antibacterial agents inspired us to conduct research on EU-modified bone cements and determine their effect on the physical properties and antibacterial activity of the obtained materials. In this work, bone cements modified with the addition of EU were obtained, and their properties, such as the doughing time, maximum temperature, and setting time, as well as the mechanical properties, were characterized. In addition, the process of EU release from the prepared materials and their antibacterial properties were investigated.

2 Materials and methods

2.1 Materials

2.1.1 Bone cement synthesis

Commercial methacrylate copolymers, K1 and K2 (Evonic, Essen, Germany); monomers, 2-hydroxyethyl methacrylate (HEMA, 97%), methyl methacrylate (MMA, 99%), and triethylene glycol dimethacrylate (TEGDM, 95%), and EU (EU, 99%); initiating system, benzoyl peroxide (BPO, >97%) and *N*,*N*-dimethylaniline (DMA, 99%) were obtained from Sigma Aldrich (St. Louis, MO, USA).

2.1.2 Release studies

Saline (FS, 0.9% NaCl, Polpharma) was purchased from the pharmacy, and pure ethanol 99.9% (EtOH, p.a.) was obtained from Chempur (Piekary Slaskie, Poland).

2.2 Methods

2.2.1 Sample preparation

The liquid phase was prepared by mixing the monomers HEMA (70 wt%), MMA (15 wt%), and TEGDM (15 wt%) with EU. EU was used in an amount of 0.5% by weight relative to the total weight of the bone cement sample (liquid + powder phase). The amount of EU was chosen to exceed the MIC of EU for the tested bacterial strains. The next step was the addition of DMA (0.50, 0.83, 1.17, and 1.63 wt%) to the previously prepared mixture of monomers and EU. The concentration of DMA was calculated in relation to the mass of the liquid phase of the cement composition, that is, the mass of monomers. The mixtures were homogenized on a mechanical shaker (MS 3 digital; IKA, Staufen im Breisgau, Germany) to obtain homogenous compositions. The powder phase was prepared separately by mixing the copolymers in a weight ratio of 80:20 (K1:K2) and then adding benzoyl peroxide (BPO) (initiator) in the following amounts: 0.3, 0.7, and 1 wt% relative to the weight of the liquid phase. The scheme of sample preparation is shown in Figure 1.

The final composition is shown in Table 1. To obtain the sample for further investigation, the powder phase was mixed with the liquid phase in a mass ratio of 1:1.25. This procedure was used to obtain all cement samples.

2.2.2 Bone cement characterization according to the ISO 5833:2002 standard

2.2.2.1 Doughing time

The powder and liquid phases were mixed, and after 1 min, the surface of the mixture was gently touched with an unpowdered, nonwater-rinsed gloved finger. It was observed whether fibers were formed between the cement and the glove as the finger left the surface. This test was repeated at 15 s intervals until the doughing time was reached. The procedure was conducted in duplicate, each time on a new, separate sample.

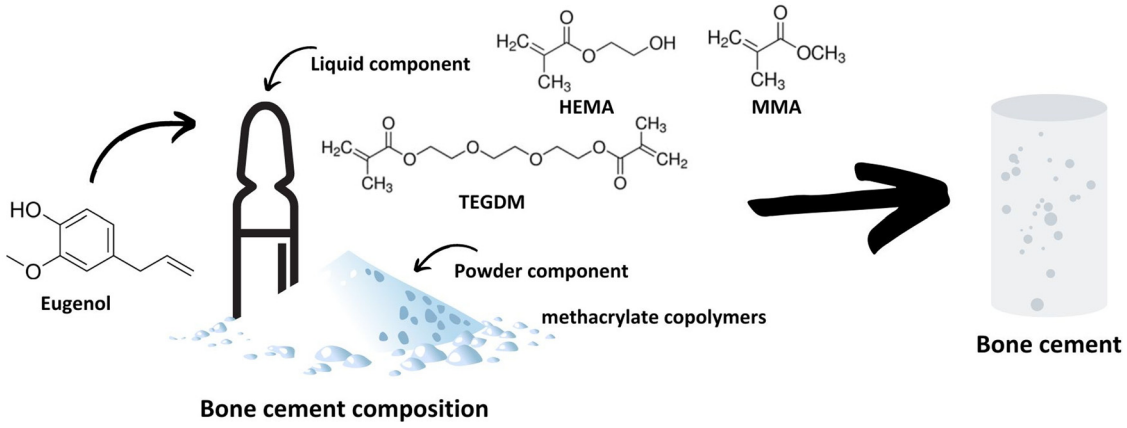

Figure 1: Scheme of bone cement sample preparation.

2.2.2.2 Temperature

The maximum temperature (T_{max}) attained by bulk, the setting temperature (T_{set}), and the setting time (t_{set}) were determined. The setting time is defined as the time to reach half the temperature increase. The powder and liquid phases were mixed and placed in the mold. The thermocouple was placed with its junction 3 ± 0.5 mm above the inner surface of the mold base, and the temperature was measured as a function of time until it began to fall. The setting temperature was calculated from Eq. (1), and the setting time was determined as the time to reach the T_{set}.

$$T_{set} = \frac{T_{max} + T_{amb}}{2} \quad (1)$$

where T_{amb} is the recorded ambient temperature, and T_{max} is the highest temperature recorded. The individual value of t_{set} for each sample was recorded with an accuracy of 5 s and then the average was calculated and rounded to the nearest 15 s.

2.2.2.3 Compressive strength

The prepared mixture of the powder and liquid phases of the bone cement was placed in cylindrical plastic (PE) molds with dimensions of 12 mm height and 6 mm diameter. Polymerization was carried out in a laboratory dryer (Memmert SF75, Memmert GmbH + Co. KG, Schwabach, Germany) for 1 h at 36.6°C and constant humidity (30%). After that time, the resulting bone cement was removed from the mold. Additionally, 24 ± 2 h after mixing the two phases (powder and liquid) of the cement, the obtained samples were subjected to a compressive strength test on a Zwick/Roell Z020 testing machine (Zwick AG, Ulm, Germany). A constant cross-head speed of 22.5 mm·min^{-1} was used. The measurement was carried out until the specimen broke and was repeated five times. The obtained results of the compressive strength and Young's modulus were recalculated using a computer program dedicated to the Zwick/Roell Z020 testing machine.

2.2.3 Microscopic examination using a scanning electron microscope (SEM)

The morphology of bone cements was studied using a JEOL 7001 F SEM (Akishima, Tokyo, Japan, SEI detector, 7 kV acceleration voltage). A gold coating was sputtered onto

Table 1: Composition of prepared non-modified bone cements

Name	Powder phase			Liquid phase			
	K1	K2	BPO (wt%)	HEMA	MMA	TEGDM	DMA (wt%)
0.3 wt% BPO and 0.5 wt% DMA	0.8	0.2	0.3	0.7	0.15	0.15	0.5
0.7 wt% BPO and 0.83 wt% DMA	0.8	0.2	0.7	0.7	0.15	0.15	0.83
0.7 wt% BPO and 1.17 wt% DMA	0.8	0.2	0.7	0.7	0.15	0.15	1.17
1 wt% BPO and 1.67 wt% DMA	0.8	0.2	1	0.7	0.15	0.15	1.67

Modified bone cements were prepared by adding 0.5 wt% of EU to each sample.

small specimens placed on a stub of the metal with carbon adhesive tape and subjected to imaging.

2.2.4 Release of EU from bone cements

The bone cements modified with EU were placed in a glass vial and immersed in 5 ml of a mixture of saline (NaCl 0.9% w/w, FS) with ethanol (50% w/w, EtOH) (FS:EtOH ratio = 1:1 v/v) at 37 ± 0.1°C. At fixed time intervals, 1 ml of the released medium was taken and replaced with an equal volume of fresh solvent, and the collected sample was analyzed by UV/Vis spectroscopy. The released amount of EU was calculated based on the calibration curve, where the extinction coefficient ε is 2194.64 ± 111.14 $M^{-1}{\cdot}cm^{-1}$ and is similar to the value reported in the literature (ε = 2563 $M^{-1}{\cdot}cm^{-1}$) [50].

2.2.5 Antibacterial properties

The prepared mixture of powder and liquid phases of bone cement was placed in cylindrical plastic (PE) molds with dimensions of 2 mm height and 8 mm diameter. The mass of the prepared discs was 0.12 ± 0.01 g. Polymerization was carried out in a laboratory dryer (Memmert SF75, Memmert GmbH + Co. KG, Schwabach, Germany) for 1 h at 36.6°C and constant humidity (30%). After that, the resulting disc-shaped bone cement sample was taken out of the mold.

Growth kinetics and viability: The disc-shaped samples after 24 h were sterilized with a UV lamp for 1 h. Each sample was then placed in a well in a 6-well plate and 7 ml of LB medium containing *Staphylococcus aureus* and *E. coil* cells with OD_{570nm} of 0.1 was added to the wells. Then, the plate was placed in the incubator at 37°C with constant shaking (230 rpm), and the OD_{570nm} of these suspensions were monitored at every 1 h interval up to 7 h and then after 24 and 48 h for bacteria assessment using a plate reader.

3 Results and discussion

For the preparation of methacrylic bone cements, we have chosen MMA, which is a commonly used monomer. Other monomers, such as HEMA and TEGDM, were used as a polymerization reaction modifier [51,52] and a cross-linking agent, respectively. Moreover, EU was used as an antibacterial agent. EU, owing to its structure, lowers the polymerization exotherm without negatively affecting the degree of conversion [53]. The polymerization of bone cements was initiated by the redox-initial system and the obtained materials were characterized to determine their parameters according to the ISO 5833:2002 standard. In addition, the release of EU from bone cements and antibacterial properties were investigated.

3.1 Doughing time

The doughing time is an important application parameter described as the time that passes from the beginning of mixing the two phases (liquid and powder) of bone cement to the moment when the bone cement dough no longer sticks to the glove surface. In other words, this is the moment when the bone cement dough can be applied during the operation. The doughing time for the tested bone cements is long, which can be caused by the effect of EU on the polymerization kinetics, as shown in the literature [53]. The average doughing time was 105 s (1.75 min) for non-modified bone cements and 240 s (4 min) for the modified bone cements. An increase in the amount of initiating system reduces the doughing time by affecting the course of polymerization, as confirmed in our earlier studies [52]. The shortest doughing time (217.5 s = 3.6 min) has modified bone cement with 1 wt% BPO and 1.17 wt% DMA (molar ratio BPO:DMA = 1:2.34); in contrast, the longest doughing time (292.5 s = 4.9 min) has bone cement with 0.7 wt% BPO and 0.5 wt% DMA (molar ratio BPO:DMA = 1:1.43). The doughing time for each of the bone cement is shown in Figure 2.

The addition of EU caused a significant increase in the doughing time. It may be related to the influence of EU on polymerization kinetics [54]. However, the requirement of

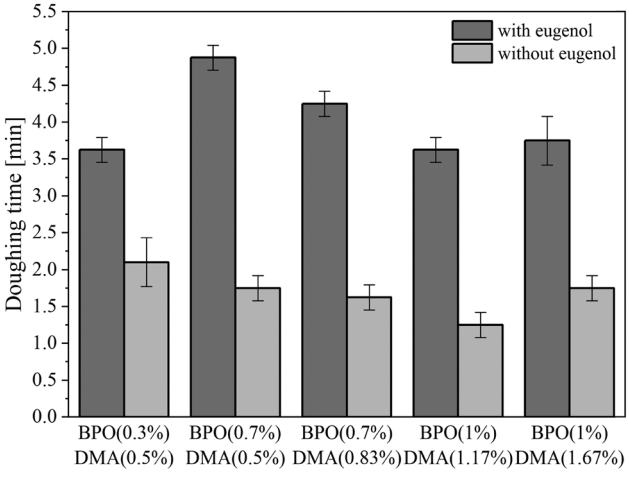

Figure 2: Doughing time (min) for the tested bone cements modified with EU (n = 4).

the ISO 5833:2002 standard indicates that the maximum doughing time is 5 min, so all types of cements tested meet this requirement [34].

3.2 Maximum curing temperature and setting time

The incorporation of EU into the bone cement resulted in a reduction of the maximum temperature during the curing process. Moreover, similar to the previously presented results of the doughing time, the setting time was also prolonged. The maximum temperature for the tested non-modified bone cements is within the range of 47.9 ± 3.4°C to 87.5 ± 0.6°C (Table 2), while for modified bone cements it is in the range of 21.2 ± 0.1°C to 23.5 ± 0.1°C (Table 2). The smallest maximum temperature was recorded for the modified bone cement with a content of 0.3 wt% BPO and 0.5 wt% DMA (molar ratio 1:3.33). Increasing the amount of initiators led to an increase in the maximum temperature and a shortening of the setting time. Lowering the curing temperature is a unique achievement because unmodified bone cements reach temperatures in the range of 45–105°C during curing, and such a high temperature can cause necrosis of the surrounding tissues [55,56]. Thus, EU-modified cement has the additional advantage of not necrotizing the surrounding tissues.

t_{set} values should be in the range of 3–15 min, according to the ISO 5833:2002 standard for the bone cements intended for dough use [34]. The three compositions with the lowest concentration of the initiating system have a t_{set} at the upper limit of the range; however, they meet this requirement [34]. As shown in previous studies [57,58], EU inhibits the polymerization of MMA. EU traps free radicals generated by BPO and, as a phenol derivative, acts as a chain-breaking radical scavenger [59]. Fujisawa and Kadoma [54] showed that the polymerization rate decreases significantly with increasing concentration of EU. Above the 0.5 mol% concentration of EU, the polymerization did not occur. This phenomenon may be due to the inhibition by some reactive products of the interaction between free radicals from BPO and EU. The results show that EU acts as a retarder against the polymerization of methacrylates. The excess EU interacts with BPO and its related compounds. The extension of the curing time may be the reason for such a decrease in T_{max}. Our study shows that increasing the amount of the initiating system resulted in a decrease in the t_{set} of the tested bone cements; however, this did not reflect on the increase of the maximum temperature for the cements modified with EU.

3.3 Compressive strength

The mechanical properties, that is, compressive strength (σ), of the bone cements with a constant amount of EU (0.5%) and various concentrations of the initiating system were studied. The samples are shown in Figure 3.

Modification of bone cements by the addition of EU resulted in obtaining materials with good compressive strength. However, Young's modulus was low for all modified bone cements. The results are shown in Figure 4a and b.

The lowest compressive strength observed for the modified bone cement with 0.3 wt% BPO and 0.5 wt% DMA (molar ratio BPO:DMA = 1:3.33) was 81.17 ± 9.94 MPa, and the Young's modulus was 113 ± 26 MPa. The highest compressive strength value of the bone cement with 1 wt% BPO and 1.17 wt% DMA (molar ratio BPO:DMA = 1:2.34) was 104.13 ± 10.61 MPa and the Young's modulus was 296 ± 44 MPa. Despite the extended curing time and lower polymerization temperature, the compressive strength of bone cements with EU was high. All tested materials meet the ISO 5833:2002 standard requirement for a minimum compressive strength (70 MPa). The addition of antibiotics in the powder form to the bone cement composition results in a decrease in the mechanical strength. The effect of antibiotic incorporation on compressive strength has been the subject of many studies. Funk *et al.* [61] showed the dependence of the antibiotic type on compressive strength. These studies showed a significant reduction in the compressive strength of bone cements

Table 2: Characteristic temperatures and setting times of the tested bone cements with EU (n = 4)

Composition	T_{max} (°C)		T_{set} (°C)		t_{set} (min)	
	EU	Control	EU	Control	EU	Control
BPO(0.3%) + DMA(0.5%)	21.20	47.88	21.00	33.15	15.00	10.45
BPO(0.7%) + DMA(0.5%)	22.85	67.64	22.03	44.87	15.00	9.50
BPO(0.7%) + DMA(0.83%)	23.10	81.30	22.33	51.98	15.00	8.10
BPO(1%) + DMA(1.17%)	23.50	78.70	22.60	50.78	7.83	5.92
BPO(1%) + DMA(1.67%)	23.05	87.5	22.28	56.4	6.50	6.5

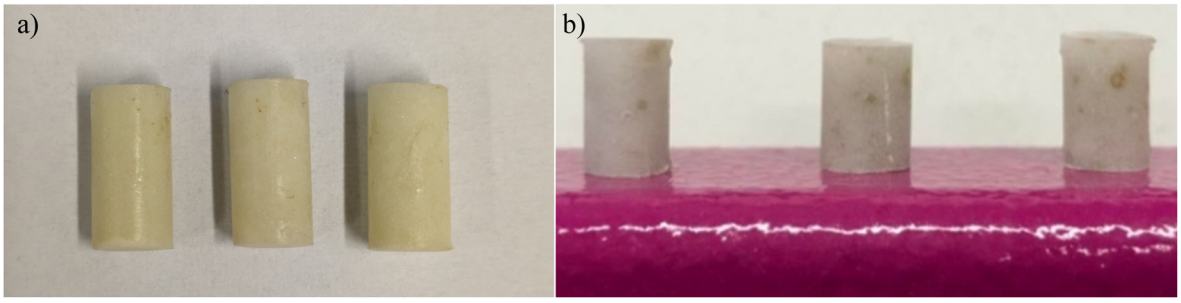

Figure 3: Bone cement samples: (a) non-modified and (b) modified with EU.

containing rifampin, while no effect was observed for samples containing vancomycin. Other studies [62] show that for low-dose ALBC (≥3.6 g of powdered antibiotic per 40 g of PMMA powder), the compressive strength does not deteriorate significantly; however, high doses (≤1 g of powdered antibiotic per 40 g of PMMA powder) of antibiotics cause significant reduction of the mechanical strength. Another factor affecting the mechanical strength is the form of the antibacterial agent. Research shows that the addition of antibiotics in the liquid form can reduce the compressive strength by even 55% (35–50 MPa) [44]. It can be related to increased porosity and changes in the material structure. The incorporation of essential oils into bone cements is not widely examined. Robu *et al.* [63] tested bone cements modified with peppermint essential oil incorporated into hydroxyapatite. The studies show a 12% decrease in compressive strength. Our studies show a decrease in the compressive strength of 7–23%. The decrease can be related to the extension of the polymerization time and the changes in the bone cement structure.

3.4 Microscopic examination by SEM

To investigate the morphology of the bone cements modified with EU, SEM images were taken (Figure 5a and b). The control sample is shown in Figure 5c and d.

The surface of the material is characterized by great roughness and porosity. In bone cement modified by EU, polymer particles can be distinguished on the surface. The change in the polymer structure could affect the release of EU and antibacterial properties and results in a high release of the active substance. In studies of the release of liquid antibiotics from bone cements [44], researchers speculate that the porosity of bone cements increases after the incorporation of antibiotics in the liquid form. According to the studies conducted by Millstein *et al.* [58], EU and EU-containing cements can alter the surface of the cured composite. Researchers make suppositions that this may be due to a chemical reaction between the composite matrix and EU; however, no hard evidence has been shown. Studies

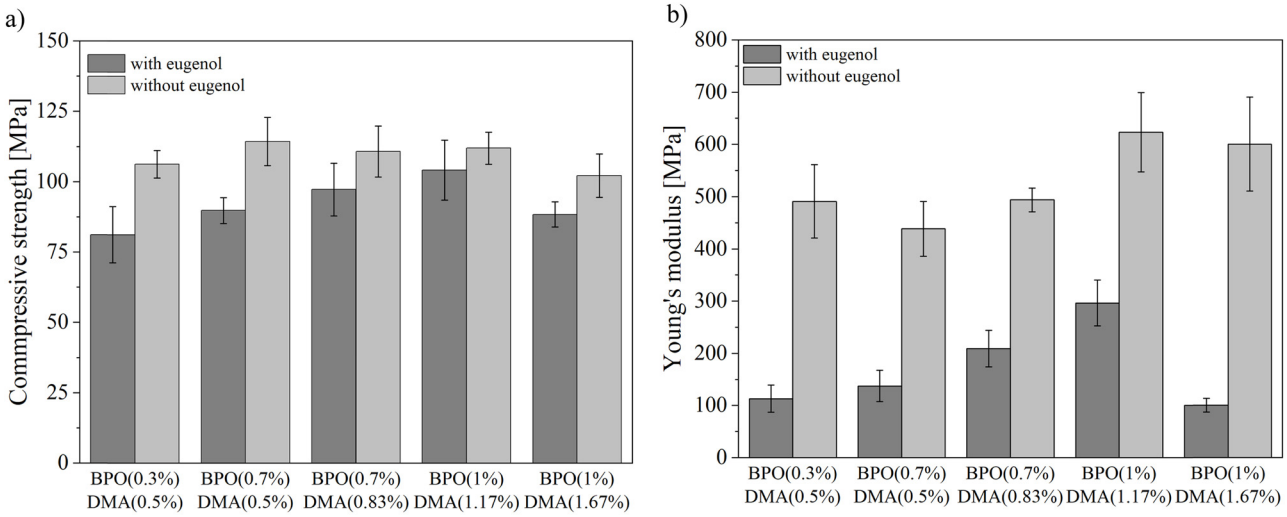

Figure 4: Mechanical properties of bone cements modified and non-modified with EU: (a) compressive strength (MPa) and (b) Young's modulus (MPa) (n = 5).

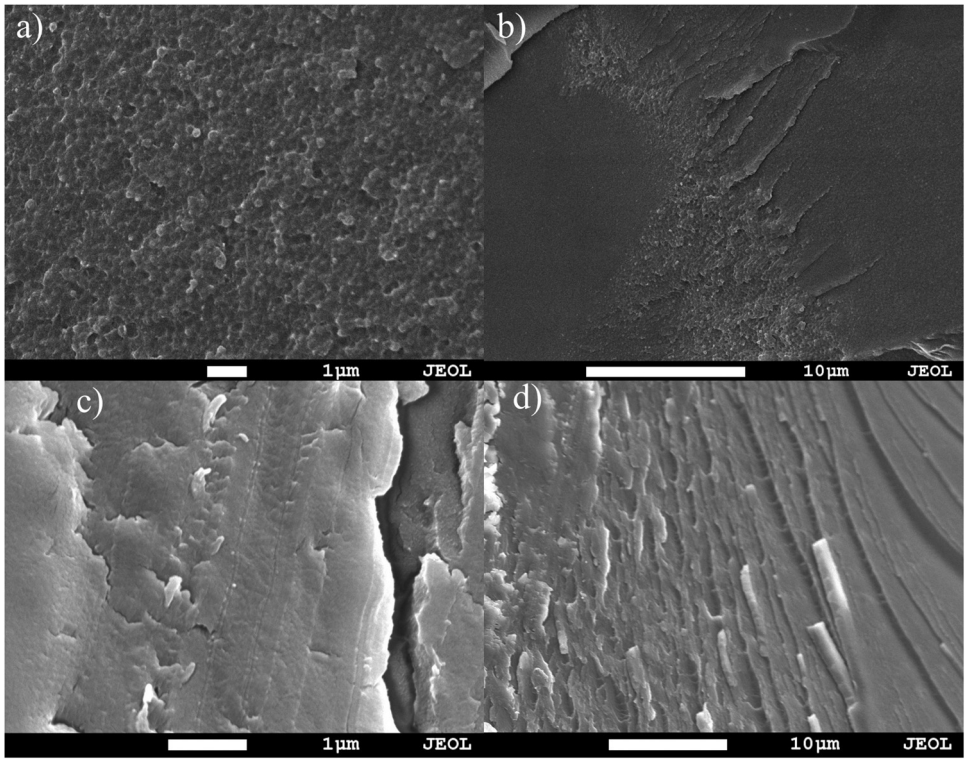

Figure 5: Morphology of selected bone cement (BPO 0.3 wt% and DMA 0.5 wt%) modified with EU (a) and (b) and non-modified (c) and (d), with different zooms.

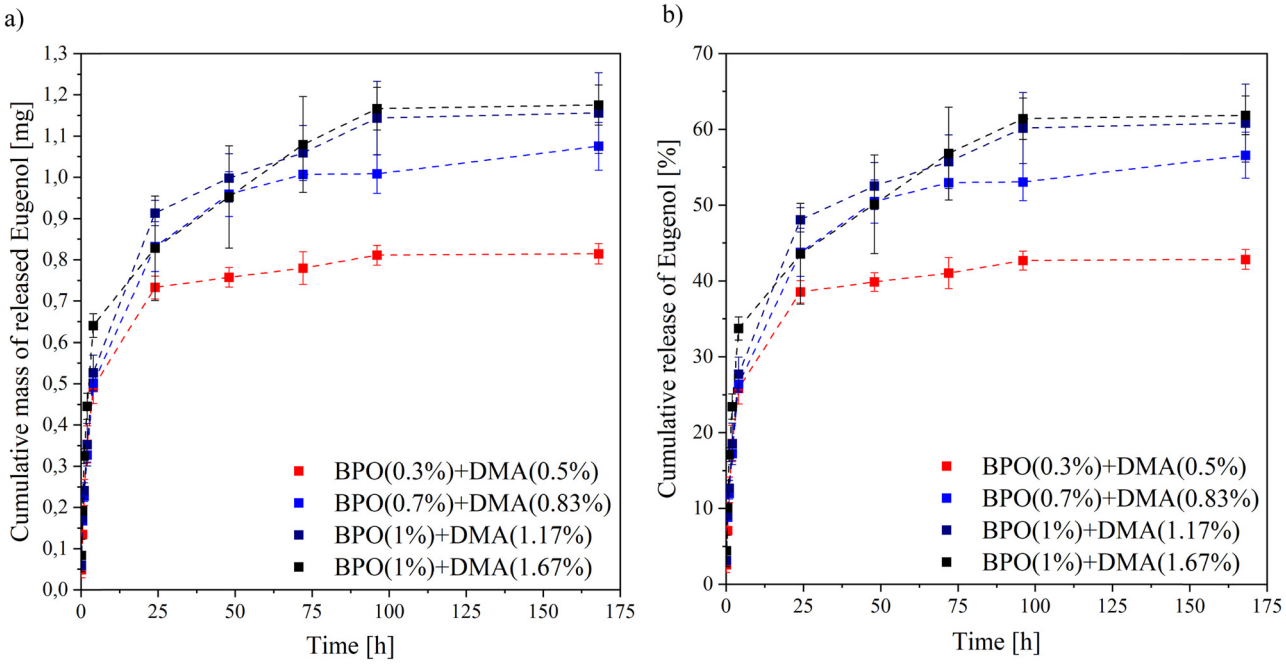

Figure 6: Release of EU from modified bone cements with different amounts of the initial system: (a) mass (mg) and (b) percent (%) (n = 3).

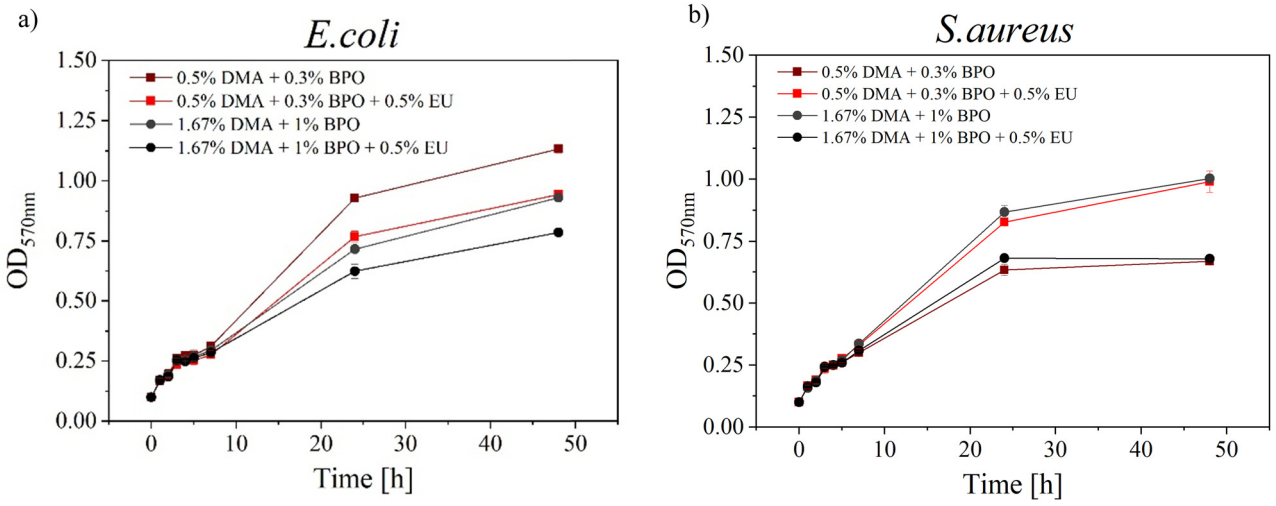

Figure 7: Influence of bone cements modified with EU on the bacterial growth of (a) *E. coli* strain and (b) *S. aureus* strain (n = 3).

on antibiotic release from the bone cement matrix show [36] that material with a structure with greater porosity improve the improve the release of active substances, which is shown in our studies.

3.5 Release of EU from bone cements

Bone cements were prepared according to the concept of sample preparation. The weight of the samples was 0.375 ± 0.005 g, and the loading efficiency was 100%, so they contained 1.9 mg of EU. The prepared bone cements were placed in FS:EtOH (ratio 1:1 v/v) solution and the samples were taken at a specific time intervals. About 1 ml of the solution over the bone cement sample was taken and filled with 1 ml of fresh FS:EtOH solution. The release of EU from bone cements differs according to the amount of the initiating system. This parameter is closely related to the structure of the polymer matrix, the surface roughness, and porosity, and those properties depend on the amount of the initiating system. The release curves are shown in Figure 6a and b.

The lowest release observed for the bone cement with 0.3 wt% BPO and 0.5 wt% DMA (molar ratio BPO:DMA = 1:3.33) was 0.814 ± 0.025 mg after 168 h, which is 42.9 ± 1.3% of the total amount of EU in the sample. The largest amounts of EU released from the bone cement with 1 wt% BPO and 1.67 wt% DMA (molar ratio BPO:DMA = 1:3.26) was 1.175 ± 0.049 mg after 168 h, which gives 61.9 ± 2.6%. Increasing the concentration of the initiating system could affect the polymer structure, *i.e.*, different crosslinking densities of the obtained bone cements and porosity of the samples, which may result in a higher release of EU. The kinetics of EU release from bone cements is characterized by a high peak of release in the first 24 h and then there is sustained release. This method of release results in the highest concentration of EU only in the first few hours, but sustained release can provide extended antibacterial activity. The kinetic release of EU from bone cements is not known; however, the release significantly exceeds the *in vivo* release from mucoadhesive tablets [60]. The reports show an increase in antibiotic release in the liquid form compared to the powder form [44], but the studies were conducted for powder antibiotics dissolved in water and not with essential oils.

3.6 Antibacterial properties – influence of EU on bacterial growth

In order to investigate the growth curve in the presence of EU-modified bone cements (disc-shaped samples), the optical density measurements at 570 nm (OD_{570nm}) of the bacterial culture were carried out. The results are presented in Figure 7a and indicate that the rate of *E. coil* bacterial growth decreases in the presence of EU (0.5% EU) for both 0.5% DMA + 0.3% BPO and 1.67% DMA + 1% BPO samples.

For *S. aureus* strain, the decreased bacterial growth was observed for samples with EU (0.5%) and remained at the same level for the pure DMA + BPO samples (Figure 7b). For both bacterial strains, delayed bacterial growth for 1.67% DMA + 1% BPO sample as compared to 0.5% DMA + 0.3% BPO can be observed. It can be concluded that the

samples with 0.5% EU are bacteriostatic for both *S. aureus* and *E. coil* strains.

4 Conclusions

Bone cements modified with EU in the amount of 0.5% meet the ISO 5833:2002 standard requirements for all parameters and are characterized by good mechanical properties: compressive strength in the range of 90–105 MPa, the high release of EU (40–63%), and exhibit bacteriostatic properties. The modification of bone cements has resulted in materials with mechanical strength comparable to that of commercial cement. Thus, the addition of EU to bone cement yields materials with the desired properties. However, the addition of EU influences the reaction kinetics and significantly extends the reaction time; therefore, there is still a need to reduce or eliminate the effect of EU on the curing process of bone cement. It is suggested that in-depth studies be performed on the possible synergistic effects of the use of EU in bone-cement materials as well as other biomaterial characterization.

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Conflict of interest: Maciej Jarzębski, who is the co-author of this article, is a current Editorial Board member of *Reviews on Advanced Materials Science*. This fact did not affect the peer-review process. The authors declare no other conflict of interest.

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