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Abstract: Bone cement based on poly(methyl methacrylate) (PMMA) powder and methyl methacrylate (MMA) liquid is a very popular biomaterial used for the fixation of artificial joints. However, there is a risk of this cement loosening from bone because of a lack of bone-bonding bioactivity. Apatite formation in the body environment is a prerequisite for cement bioactivity. Additionally, suppression of infection during implantation is required for bone cements to be successfully introduced into the human body. In this study, we modified PMMA cement with □-methacryloxypropyltrimetoxysilane and calcium acetate to introduce bioactive properties and 2-(tert-butylamino)ethyl methacrylate (TBAEMA) to provide antibacterial properties. The long-term antibacterial activity is attributed to the copolymerization of TBAEMA and MMA. As the TBAEMA content increased, the setting time increased and the compressive strength decreased. After soaking in simulated body fluid, an apatite layer was detected within 7 d, irrespective of the TBAEMA content. The cement showed better antibacterial activity against Gram-negative E. Coli than Gram-positive bacteria; however, of the Gram-positive bacteria investigated, B. subtilis was more susceptible than S. aureus.

Preparation of bioactive and antibacterial PMMA-based bone cement by modification with quaternary ammonium and alkoxysilane

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Abstract

Bone cement based on poly(methyl methacrylate) (PMMA) powder and methyl methacrylate (MMA) liquid is a very popular biomaterial used for the fixation of artificial joints. However, there is a risk of this cement loosening from bone because of a lack of bone-bonding bioactivity. Apatite formation in the body environment is a prerequisite for cement bioactivity. Additionally, suppression of infection during implantation is required for bone cements to be successfully introduced into the human body. In this study, we modified PMMA cement with γ-methacryloxypropyltrimetoxysilane and calcium acetate to introduce bioactive properties and 2-(tert-butylamino)ethyl methacrylate (TBAEMA) to provide antibacterial properties. The long-term antibacterial activity is attributed to the copolymerization of TBAEMA and MMA. As the TBAEMA content increased, the setting time increased and the compressive strength decreased. After soaking in simulated body fluid, an apatite layer was detected within 7 d, irrespective of the TBAEMA content. The cement showed better antibacterial activity against Gram-negative *E. Coli* than Gram-positive bacteria; however, of the Gram-positive bacteria investigated, *B. subtilis* was more susceptible than *S. aureus*.

Keywords: PMMA bone cement, 2-(tert-butylamino)ethyl methacrylate, mechanical property, apatite formation, antibacterial activity

1. Introduction

Bone cement comprising poly(methyl methacrylate) (PMMA) powder and methyl methacrylate (MMA) liquid is a popular bone-repair material used in orthopedic surgery for fixing artificial joints and curing osteoporosis-related fractures.[1,2] The fixation strength of PMMA cement to bone depends primarily on mechanical interlocking.[3] Several strategies have been employed to improve cement–bone interactions, for example PMMA bone cement is often mixed with bioactive ceramic powders such as sintered hydroxyapatite and glass-ceramic A-W.[4,5] However, large amounts of the ceramic are required to make the cement bioactive as the added particles make limited contact with body fluid.

Bioactive ceramics described above generally achieve the bone-bonding as follows. Bone-like apatite layer is formed on the ceramics via chemical reaction with body fluid. Then, adsorption, proliferation and differentiation of osteoblast are occurred. Consequently, bone matrix is calcified to fill the gap between the bone tissue and the implants.[6] Inspired by the bone-bonding mechanism of bioactive ceramics, we previously reported that PMMA cement exhibited apatite-forming properties in simulated body fluid (SBF) when it was modified with an alkoxysilane, such as γ-methacryloxypropyltrimethoxysilane (MPS), and water-soluble calcium salts.[7,8] MPS contributes to silanol (Si-OH) group formation and is able to induce heterogeneous apatite nucleation, which is accelerated by released Ca^{2+}. This type of apatite formation in SBF is required for materials to show bioactivity.[9] Novel nanomaterials such as graphene and boron nitride nanotube are attractive for improvement of biological compatibility of PMMA and calcium phosphate bone cements.[10,11]

Another challenge facing PMMA bone cement is bacterial infection as a result of it being implanted in the human body.[12] For this purpose, PMMA cements loaded with various antibiotics

such as gentamicin and vancomycin have been developed and some of them have been already commercialized[13,14]. In order to extend the antibacterial spectra to resistant bacteria, viruses and so on, non-antibiotic PMMA cements have been recently developed. For example, antibacterial PMMA cements loaded with silver and gold nanoparticles have been proposed.[15,16] Graphene and iron ions are also known as novel antibacterial chemical species.[17,18] In addition, control in release profiles of antibacterial agents introduces a challenge. It is reported that sustained release of antibiotics from the cements can be achieved by incorporation of carboxyl acid-functionalized block copolymers[19] and mesoporous silica[20], and by encapsulation of the antibiotics with various inorganic and organic compounds[21].

Quaternary ammonium has good antibacterial activity against a wide range of microorganisms, while exhibiting only low toxicity in mammals.[22] Lenoir *et al.*[23] assumed that the positively charged amino groups replace divalent cations of the outer membrane, which leads to membrane disruption. Copolymerization of MMA and a monomer containing antibacterial quaternary ammonium is expected to lead to long-term antibacterial activity. Abid *et al.* prepared antibacterial PMMA cement by copolymerization of a quaternary ammonium dendrimer.[24] As a result, compressive strength of the cement was around 50 MPa and the prepared cement showed antibacterial activity against both Gram-positive and Gram-negative bacteria. The cement is expected to inhibit infection in prosthesis and surgery. However, neither evaluation of bioactivity nor improvement of the bioactivity was attempted.

In this study, PMMA cement was modified with 2-(tert-butylamino)ethyl methacrylate (TBAEMA, $H_2C=C(CH_3)CO_2CH_2CH_2NHC(CH_3)_3$), MPS, and calcium acetate. The assumed chemical structure is schematically illustrated in Fig. 1. Its bioactivity was assessed *in vitro* using

SBF and its antibacterial activity was evaluated by culturing two Gram-positive bacteria and a Gram-negative bacterium. The obtained results are discussed in terms of TBAEMA content.

2. Materials and Methods

2.1. Materials

PMMA powder (MB-4C) was procured from Sekisui Plastics Co., Ltd. (Tokyo, Japan). Calcium acetate monohydrate, MMA, and N,N-dimethyl-*p*-toluidine (NDT) were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). TBAEMA was purchased from Sigma-Aldrich Co. LLC (Missouri, USA). Benzoyl peroxide (BPO), LB Lennox culture medium and the chemicals for the preparation of SBF were acquired from Nacalai Tesque Inc. (Kyoto, Japan). MPS was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). $Ca(CH_3COO)_2$ was dried at 220°C and BPO was recrystallized from ethanol. The other chemicals were of analytical grade and were used without further purification. Commercial gentamicin-loaded antibacterial cement (Cobalt G-HV, Zimmer Biomet, IN, USA) was used as a reference. This type of cement contains 2% of the gentamicin in powder component.

2.2. Cement preparation

The PMMA based bone cement included two components: the powder source and the liquid source. The powder source was composed of PMMA, $Ca(CH_3COO)_2$, and BPO. The liquid source comprised MMA, MPS, TBAEMA, and NDT. The detailed composition of each component is shown in Table 1. The mixing ratio of powder/liquid was 1/0.5 (g/g). The temperature and relative

humidity during mixing were maintained at 23 ± 2°C and 50±10%, respectively. The paste was injected into a cylindrical or rectangular polypropylene mold for setting. TBAEMA/(MMA+ TBAEMA) mass ratio of the cements was ranged from 0 to 40%, because addition of larger amount of TBAEMA made the setting of the cement quite difficult.

2.3. Setting time and temperature measurement

A mixed paste of 1.5 g of cement was used to determine the setting time. A weight of 300 g was loaded onto the mixed paste with a Vicat needle apparatus (A-004, JAPAN MECC Co., Ltd., Tokyo, Japan) with a cross section of 1 mm^2. The setting time was defined as the time when the trace of the Vicat needle did not remain on the surface of the cement. The test was repeated five times for each cement. Temperature change of the mixed paste was monitored by a temperature data logger attached with a Pt resistance temperature detector (TR-81, T&D Corporation, Nagano, Japan). Five specimens were tested for each cement composition.

2.4. Mechanical properties

Cylindrical samples 6 mm in diameter and 12 mm in height were used for the compressive strength measurements. All of the specimens were subjected to a compressive load with a cross-head speed of 1 mm/min controlled by a universal testing machine (Autograph AG, Shimadzu Co., Kyoto, Japan) until fracture occurred. The compressive strength was calculated from the fracture load and the cross-sectional area of the samples. Five specimens were tested for each cement composition.

2.5. Bioactivity test in SBF

The bioactivity of the obtained PMMA bone cement was evaluated *in vitro* in terms of apatite formation on the surface in SBF (Na^+ 142.0, K^+ 5.0, Mg^{2+} 1.5, Ca^{2+} 2.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5 mM). The SBF was prepared by successively dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, and Na_2SO_4 in ultrapure water. The pH of the SBF was adjusted to 7.40 using 1 M HCl and 50 mM tris(hydroxymethyl)aminomethane. Details of the SBF preparation were reported previously.[25] The cements were shaped into rectangular pieces with dimensions of 10 × 10 × 1 mm^3 and then immersed in polystyrene bottles containing 35 mL of SBF at 36.5°C. After soaking for a certain period of time, the cements were taken out, rinsed with pure water three times, and dried in an oven at 65°C for 24 h.

2.6. Characterization

The surfaces of the cements were characterized by thin-film X-ray diffractometery (TF-XRD, MXP3V, MAC Science Ltd., Yokohama, Japan), Fourier-transform infrared spectroscopy (FT-IR, FT/IR-6100, JASCO Co., Tokyo, Japan) and scanning electron microscopy (S-3500N, Hitachi Co., Tokyo, Japan). In the TF-XRD experiments, the incident beam was fixed at 1° to the surface of each substrate and the scan rate was $0.02° \cdot s^{-1}$. In FT-IR measurements, an attenuated total reflection (ATR) attachment with a diamond crystal was used. All of the cement specimens were coated with a thin film of Au-Pd using an ion sputter coater (E-101, Hitachi Co., Tokyo, Japan) prior to SEM observation. To determine the chemical structure of the quaternary ammonium

component leached from the cements, a cylindrical specimen 6 mm in diameter and 12 mm in height containing 40 % TBAEMA was soaked in 3 mL of ultrapure water for 1 d. The solution was analyzed by mass spectrometry in positive ion mode (JMS-SX102A, JEOL Ltd., Tokyo, Japan).

2.7. Antibacterial activity test

Cylindrical cement samples, 6 mm in diameter and 12 mm in height, modified with different amounts of TBAEMA were soaked in 5 mL of Luria-Bertani (LB) Lennox culture medium (10 g of tryptone, 5 g of yeast extract and 5 g of NaCl for 1 L)[26] and sterilized using an autoclave (BS-245, TOMY SEIKO Co., Ltd., Tokyo Japan) at 120°C for 20 min. After cooling to room temperature, the samples were transferred to test tubes and shaken in a constant temperature incubator (MM-10, TAITEC Co., Saitama, Japan) for a certain period (1 or 10 d) at 37°C and 120 rpm. After removing the cements, two kinds of Gram-positive bacteria—*Staphylococcus aureus* (*S. aureus*) strain 209P and *Bacillus subtilis* (*B. subtilis*) strain ATCC 6633—and the Gram-negative *Escherichia coli* (*E. coli*) strain NIHJ, were individually inoculated to be 0.05 of OD620 as the initial cell turbidity of each bacterial strain, corresponding to 1–5 × 10^7 CFU/mL. The mixtures were then incubated at 37°C and 120 rpm for 4 or 8 h.[27–29] The turbidity and transmittance in the tubes were measured using a spectrometer (miniphoto518R, TAITEC Co., Saitama, Japan) at 620 nm. At least three samples were tested for each composition.

3. Results

Figure 2 shows the setting time of cements modified with different amounts of TBAEMA. The setting time increased with increasing TBAEMA content, and satisfied the requirement of ISO 5833 for all TBAEMA contents of 10% or less. The cement did not set at ambient temperature when the TBAEMA content exceeded 40%. If the setting time is too short, handling by a medical doctor becomes quite difficult. In the opposite case, there is a risk of mixing of the cement paste with surrounding body fluid including blood before the setting, leading to degradation of the mechanical strength. Therefore, moderate setting rate regulated by ISO is important. Maximum temperature of the cement paste after the mixing is shown in Fig. 3. The temperature decreased with increase in TBAEMA content. It did not exceed the maximum temperature of conventional bone cements.

Figure 4 shows the compressive strength of cements modified with different amounts of TBAEMA. The compressive strength decreased with increasing TBAEMA content, and satisfied the requirement of ISO 5833 for TBAEMA contents of 5% or less.

Figure 5 shows FT-IR ATR spectra of the cements with TBAEMA content of 0 and 10%. In addition to a lot of bands derived from MMA, MPS and calcium acetate, C-N band derived from TBAEMA was observed around 1400 cm^{-1} at TBAEMA content of 10%.[30-34]

Figure 6 shows SEM images of the surfaces of cements modified with different amounts of TBAEMA, as well as Cobalt G-HV cement, before and after soaked in SBF for 7 d. A lot of spherical PMMA particles were observed before soaking. On the other hand, assemblies of fine particles covered the surfaces of all of the modified cements. Such particles were also partially observed on the Cobalt G-HV cement. The particle morphology was similar to that of apatite formed in SBF.[35] The TF-XRD patterns of the same samples are shown in Fig. 7. Broad peaks

assigned to hydroxyapatite (JCPDS#09-0432) with low crystallinity were detected at approximately 26° and 32° for all of the modified cements, but not for Cobalt G-HV cement. Peaks assigned to monoclinic ZrO_2 (JCPDS#83-0944), added as an X-ray opacifier, were detected for the Cobalt G-HV cement. We found that the addition of TBAEMA had little influence on apatite formation.

Figure 8 shows the turbidity of the LB culture medium after soaking the TBAEMA modified cements for 1 or 10 d and subsequently incubating the extract with various bacteria for 4 or 8 h. A decrease in the turbidity corresponded to a decrease in the concentration of bacteria. The turbidity tended to decrease as the amount of TBAEMA in the cement increased, regardless of the extraction time or culture time. The turbidity increased slightly when the bacterial culture time was increased from 4 h to 8 h. When the cements containing 40% TBAEMA were extracted for 10 d, *B. subtilis* was completely killed. However, *E. coli* was more susceptible as it was completely killed at 30%. The antibacterial properties varied in the order of susceptibility: *E. coli*>*B. subtilis*>*S. aureus* for samples extracted for 10 d.

Figure 9 shows the mass spectrum of ultrapure water after soaking the 40% TBAEMA modified cement for 1 d. Peaks assigned to protonated $C_6H_{15}NO$ and $C_{10}H_{19}NO_2$ were detected at m/z 118.12 and 186.15, respectively. $C_6H_{15}NO$ and $C_{10}H_{19}NO_2$ correspond to an aminoalcohol formed by hydrolysis of TBAEMA and the TBAEMA monomer, respectively.

4. Discussion

We found that PMMA cement modified with MPS and TBAEMA showed self-hardening properties at certain compositions (Figs. 2 and 5). However, the compressive strength tended to

decrease as TBAEMA content increased (Fig. 4). According to the mass spectrum in Fig. 9, the amount of TBAEMA (m/z=186.15) eluted was much larger than that of MMA (m/z=100.11). Erol *et al.*[36] reported that the monomer reactivity ratio of (2 - oxo - 2 - tert - butylamino)ethylene methacrylate containing amino groups is lower than that of styrene. Paleari *et al.*[37] synthesized a copolymer of TBAEMA and MMA for use as a denture base and found that the decrease in the degree of polymerization and associated residual monomer serve as a plasticizer, thereby reducing the strength of the copolymer. Therefore, the residual monomer may result in reduced mechanical strength. This is supported by the results in Fig. 3 showing reduction of polymerization temperature at high TBAEMA content. It has been reported that the mechanical strength of PMMA bone cement is improved by the incorporation of cross-linking.[38] Additionally, since the polymerization initiators and accelerators used are optimized for MMA, further studies are needed regarding the improvement of radical polymerization of TBAEMA in future work.

The apatite-forming properties of the cements in SBF were almost the same regardless of the amount of TBAEMA added (Figs. 6 and 7). It is known that the ability of amino groups to form apatite is not as great as those of carboxyl or phosphate groups.[39] Nevertheless, the addition of TBAEMA did not reduce apatite formation. This is probably the result of sufficient Ca^{2+} being released from the cement. Interestingly, commercial Cobalt G-HV cement also formed a small amount of apatite (Fig. 6). Because PMMA cement itself does not show apatite formation,[7] it is thought that the added gentamicin contributes to apatite formation in the case of Cobalt G-HV. Although the detailed mechanism is not currently clear, gentamicin has the ability to form complexes with various cations[40] and anions;[41] therefore, it is thought that the complex formed in SBF induced apatite formation. Also, there is possibility of synergy effect of zirconia added as

opacifier, since a kind of hydrated zirconia has apatite-forming ability.[42] The above observation may lead to the design of novel gentamicin-based materials with both bioactivity and antibacterial activity.

We found that the prepared cement exhibited antibacterial activity even in the form of an extract (Fig. 8). The cement with TBAEMA content of 5% had the desired setting behavior and mechanical properties. Its antibacterial activity against *E. coli* and *S. aureus* was lower than that of the previously reported cement with quaternary ammonium dendrimer of 5%, that exhibited comparable antibacterial activity to commercial antibacterial bone cement.[24] This difference is attributed to differences in the culture methods. In the previous study, bacteria were cultivated with the powdered cement; however, in this study they were cultured with the extracts of the set cements. The condition of the present study would reproduce the actual clinical environment more accurately. It is therefore expected that the antibacterial activity of the present cement would be enhanced if the bacteria made direct contact with the cement. It was also found from Fig. 8 that even the cements without TBAEMA showed suppression of the bacteria growth to some extent. The addition of NaCl or $CaCl_2$ into culture media is reported to be more effective for growth inhibition of *L. pentosus* and *S. cerevisiae* than KCl or $MgCl_2$.[43] It is therefore expected that the released Ca^{2+} contributes to the antibacterial activity of the incorporated quaternary ammonium.

Gram-negative *E. coli* showed greater susceptibility to the cement extracts than the Gram-positive bacteria *S. aureus* and *B. subtilis*. This difference can be explained by the properties of the outer membranes of the bacteria.[44] The bactericidal efficacy of poly(TBAEMA) depends on a high surface density of positive charge. Gram-negative bacteria are more negatively charged on the surface than Gram-positive bacteria and therefore interact with poly(TBAEMA)-based cements more strongly.

Of the tested Gram-positive bacteria, *B. subtilis* was more susceptible to the cement extracts than *S. aureus*. Although the tendency is not consistent, previous studies using polymers with quaternary ammonium groups have shown differences in sensitivity for different bacteria.[34,36] In this study—owing to the fact that the eluted monomers and hydrolysis products contribute to the antibacterial activity—the difference may be caused by the morphology of the bacteria. Namely, *S. aureus* has spherical morphology,[45] whereas *B. subtilis* is more wrinkled and uneven[46]. Additionally, the former easily form a cluster. Therefore, the specific surface area and number of available adsorption sites would be larger for *B. subtilis* than for *S. aureus*. It has also been reported that antibacterial activity is governed by molecular weight.[34] Increasing the molecular weight of the cement is important for enhancing the mechanical properties. Therefore, the relationship between the degree of polymerization and the antibacterial activity should be further investigated.

5. Conclusion

PMMA cement exhibiting both bioactivity and antibacterial activity was obtained by chemical modification with quaternary ammonium, alkoxysilane, and water-soluble calcium salt. The cement showed greater antibacterial activity against *E. Coli*, a Gram-negative bacterium, as compared to two Gram-positive bacteria, *S. aureus* and *S. subtilis*. The findings demonstrate the potential to develop a novel bioactive bone cement with sustained antibacterial activity and high mechanical strength through appropriate control of the degree of polymerization and cross-linking structure.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

References

1. Harper EJ. Bioactive bone cements. *Proc Inst Mech Eng H: J Eng Med* 1998; 212: 113-120.
2. Breusch SJ, Aldinger PR, Thomsen M, Ewerbeck V, Lukoschek M. Anchoring principles in hip endoprostheses. I: Prosthesis stem. *Der Unfallchirurg* 2000; 103: 918-931.
3. Ravaglioli A, Krajewski, A. *Bioceramics: Materials·Properties·Applications*. Netherlands: Springer; 1992. pp. 314-341.
4. Kobayashi M, Nakamura T, Okada Y, Fukumoto A, Furukawa T, Kato H, Kokubo T, Kikutani T. Bioactive bone cement: Comparison of apatite and wollastonite containing glass‐ceramic, hydroxyapatite, and β-tricalcium phosphate fillers on bone-bonding strength. *J Biomed Mater Res* 1998; 42: 223-237.
5. Mousa WF, Kobayashi M, Shinzato S, Kamimura M, Neo M., Yoshihara S, Nakamura T. Biological and mechanical properties of PMMA-based bioactive bone cements. *Biomaterials* 2000; 21: 2137-2146.

6. Hench LL. The story of Bioglass®. *J Mater Sci: Mater Med* 2006; 17: 967-978.
7. Ohtsuki C., Miyazaki T., Kyomoto M., Tanihara M., Osaka, A. Development of bioactive PMMA-based cement by modification with alkoxysilane and calcium salt. *J Mater Sci: Mater Med* 2001; 12: 895-899.
8. Miyazaki T, Ohtsuki C, Kyomoto M, Tanihara M, Mori A, Kuramoto K. Bioactive PMMA bone cement prepared by modification with methacryloxypropyltrimethoxysilane and calcium chloride. *J Biomed Mater Res* 2003; 67A: 1417-1423.
9. Kokubo T, Kim HM, Kawashita M. Novel bioactive materials with different mechanical properties. *Biomaterials* 2003; 24: 2161-2175.
10. Sharma R, Kapusetti G, Bhong SY, Roy P, Singh SK, Singh S, Balavigneswaran CK, Mahato KK, Ray B, Maiti P, Misra N. Osteoconductive amine-functionalized graphene–poly(methyl methacrylate) bone cement composite with controlled exothermic polymerization. *Bioconjugate Chem* 2017; 28: 2254-2265.
11. Rau JV, Fosca M, Fadeeva IV, Kalay S, Culha M, Raucci MG, Fasolino I, Ambrosio L, Antoniac IV, Uskoković V. Tricalcium phosphate cement supplemented with boron nitride nanotubes with enhanced biological properties. *Mater Sci Eng C* 2020; 114: 111044.
12. Elson RA, Jephcott AE, McGechie DB, Verettas D. Bacterial infection and acrylic cement in the rat. *J Bone Joint Surg Br* 1977; 59: 452-457.
13. Kühn KD. *Bone Cements*. Netherlands: Springer; 2000. pp. 149-225.
14. Gandomkarzadeh M, Moghimi HR, Mahboubi A. Evaluation of the Effect of Ciprofloxacin and Vancomycin on Mechanical Properties of PMMA Cement; a Preliminary Study on Molecular Weight. *Sci Rep* 2020; 10: 3981.

15. Alt V, Bechert T, Steinrücke P, Wagener M, Seidel P, Dingeldein E, Domann E, Schnettler R. An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement. *Biomaterials* 2004; 25: 4383-4391.
16. Russo T, Gloria A, De Santis R, D'Amora U, Balato G, Vollaro A, Oliviero O, Improta G, Triassi M, Ambrosio L. Preliminary focus on the mechanical and antibacterial activity of a PMMA-based bone cement loaded with gold nanoparticles. Bioactive Mater 2017; 2: 156-161.
17. Uskoković V, Graziani V, Wu VM, Fadeeva IV, Fomin AS, Presniakov IA, Fosca M, Ortenzi M, Caminiti R, Rau JV. Gold is for the mistress, silver for the maid: Enhanced mechanical properties, osteoinduction and antibacterial activity due to iron doping of tricalcium phosphate bone cements. *Mater Sci Eng C* 2019; 94: 798-810.
18. Zapata MEV, Ruiz Rojas LM, Mina Hernández JH, Delgado-Ospina J, Tovar CDG. Acrylic bone cements modified with graphene oxide: mechanical, physical, and antibacterial properties. *Polymers* 2020; 12: 1773.
19. Liang ZC, Yang C, Ding X, Hedrick JL, Wang W, Yang YY. Carboxylic acid-functionalized polycarbonates as bone cement additives for enhanced and sustained release of antibiotics. *J Control Release* 2021; 329: 871-881.
20. Letchmanan K, Shen SC, Ng WK, Kingshuk P, Shi Z, Wang W, Tan RBH. Mechanical properties and antibiotic release characteristics of poly(methyl methacrylate)-based bone cement formulated with mesoporous silica nanoparticles. *J Mech Behav Biomed Mater* 2017; 72: 163-170.
21. Oungeun P, Rojanathanes R, Pinsornsak P, Wanichwecharungruang S. Sustaining antibiotic release from a poly(methyl methacrylate) bone-spacer. *ACS Omega* 2019; 4: 14860-14867.

22. Thölmann D, Kossmann B, Sosna F. Polymers with antimicrobial properties. *Eur Coat J* 2003; 1: 16-33.
23. Lenoir S, Pagnoulle C, Galleni M, Compère P, Jérôme R, Detrembleur C. Polyolefin matrixes with permanent antibacterial activity: preparation, antibacterial activity, and action mode of the active species. *Biomacromolecules* 2006; 7: 2291-2296.
24. Abid CK, Jain S, Jackeray R, Chattopadhyay S, Singh H. Formulation and characterization of antimicrobial quaternary ammonium dendrimer in poly(methyl methcarylate) bone cement. *J Biomed Mater Res B Appl Biomater* 2017; 105: 521‑530.
25. Cho SB, Nakanishi K, Kokubo T, Soga N, Ohtsuki C, Nakamura T, Kitsugi T, Yamamuro T. Dependence of apatite formation on silica gel on its structure: effect of heat treatment. *J Am Ceram Soc* 1995; 78: 1769-1774.
26. Sezonov G, Joseleau-Petit D, D'Ari R. Escherichia coli physiology in Luria-Bertani broth. *J Bacteriol* 2007; 189: 8746-8749.
27. Orhan DD, Özçelik B, Özgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res 2010;165:496-504.
28. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomater* 2008; 4: 707-716.
29. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of fill canal, sealapex, mineral trioxide aggregate, Portland cement and endorez. *Int Endod J* 2005; 38: 539-543.
30. Guo Z, Pereira T, Choi O, Wang Y, Hahn HT. Surface functionalized alumina nanoparticle filled polymeric nanocomposites with enhanced mechanical properties. *J Mater Chem* 2006; 16: 2800-2808.

31. Musumeci AW, Frost RL, Waclawik ER. A spectroscopic study of the mineral paceite (calcium acetate). *Spectrochim Acta A* 2007; 67: 649-661.
32. Duan G, Zhang C, Li A, Yang X, Lu L, Wang X. Preparation and characterization of mesoporous zirconia made by using a poly (methyl methacrylate) template. *Nanoscale Res Lett* 2008; 28: 118-122.
33. Namouchi F, Smaoui H, Fourati N, Zerrouki C, Guermazi H, Bonnet JJ. Investigation on electrical properties of thermally aged PMMA by combined use of FTIR and impedance spectroscopies. *J Alloy Compd* 2009; 469: 197-202.
34. Zuo H; Wu D, Fu R. Preparation of antibacterial poly (methyl methacrylate) by solution blending with water‐insoluble antibacterial agent poly [(tert‐buty1amino) ethyl methacrylate]. *J Appl Polym Sci* 2012; 125: 3537-3544.
35. Kokubo T, Ito S, Huang ZT, Hayashi T, Sakka S, Kitsugi T, Yamamuro T. Ca,P-rich layer formed on high-strength bioactive glass-ceramic A-W. *J Biomed Mater Res* 1990; 24: 331‐343.
36. Erol I, Yavuz F. Copolymers of (2‐oxo‐2‐tert‐butylamino) ethylene methacrylate and styrene: synthesis, characterization and monomer reactivity ratios. *Polym Int* 2005; 54: 506-512.
37. Paleari AG, Marra J, Pero AC, Rodriguez LS, Ruvolo-Filho A, Compagnoni MA. Effect of incorporation of 2-tert-butylaminoethyl methacrylate on flexural strength of a denture base acrylic resin. *J Appl Oral Sci* 2011; 19: 195‐199.

38. Deb S, Vazquez B, Bonfield W. Effect of crosslinking agents on acrylic bone cements based on poly(methylmethacrylate). *J Biomed Mater Res* 1997; 37: 465-473.

39. Tanahashi M, Matsuda T. Surface functional group dependence on apatite formation on self-assembled monolayers in a simulated body fluid. *J Biomed Mater Res* 1997; 34: 305-315.

40. Mishra P, Sharma RK. Gentamicin complexes of cobalt(II), nickel(II), cadmium(II) and tin(II). *Main Group Chem* 2008; 7: 83-95.

41. Bohner M, Lemaître J, Merkle HP, Gander B. Control of gentamicin release from a calcium phosphate cement by admixed poly(acrylic acid). *J Pharm Sci* 2000; 89: 1262-1270.

42. Uchida M, Kim HM, Kokubo T, Miyaji F, Nakamura T. Bonelike apatite formation induced on zirconia gel in a simulated body fluid and its modified solutions. *J Am Ceram Soc* 2001; 84: 2041-2044.

43. Jain A, Duvvuri LS, Farah S, Beyth N, Domb AJ, Khan W. Antimicrobial polymers. *Adv Healthcare Mater* 2014; 3: 1969-1985.

44. Bautista-Gallego J, Arroyo-López FN, Durán-Quintana MC, Garrido-Fernandez A. Individual effects of sodium, potassium, calcium, and magnesium chloride salts on Lactobacillus pentosus and Saccharomyces cerevisiae growth. *J Food Prot* 2008; 71: 1412-1421.

45. Shimoda M, Ohki K, Shimamoto Y, Kohashi O. Morphology of defensin-treated Staphylococcus aureus. *Infect Immun* 1995; 63: 2886-2891.

46. Reeve JN, Mendelson NH. Cell morphology of Bacillus subtilis: the effect of genetic background on the expression of a rod - gene. *Mol Gen Genet* 1972; 119: 11-26.

Table 1 Composition of the powder and liquid phases of the modified cements

Powder (per 1 g) / mg			Liquid (per 0.5 g) / mg			
PMMA	BPO	Calcium acetate	MMA	TBAEMA	MPS	NDT
777	29	194	x	(486-x)	9.7	3.9

Figure captions

Figure 1 Assumed chemical structure of the cement prepared in this study.

Figure 2 Setting time of cements modified with different amounts of 2-(tert-butylamino)ethyl methacrylate (TBAEMA).

Figure 3 Maximum temperature of the cement paste after the mixing.

Figure 4 Compressive strength of cements modified with different amounts of TBAEMA.

Figure 5 FT-IR ATR spectra of the cements with TBAEMA content of 0 and 10%.

Figure 6 SEM images of the surfaces of cements modified with different amounts of TBAEMA and Cobalt G-HV cement before and after soaked in SBF for 7 d.

Figure 7 TF-XRD patterns of the surfaces of cements modified with different amounts of TBAEMA and Cobalt G-HV cement. All samples were soaked in SBF for 7 d.

Figure 8 Turbidity of LB culture medium after soaking the TBAEMA modified cements for 1 or 10 d and subsequently incubating the extracts with various bacteria for 4 or 8 h. * and ns denote $p<0.05$ and no significance, respectively.

Figure 9 Mass spectrum of ultrapure water after soaking the cement with 40% TBAEMA content for 1 d.

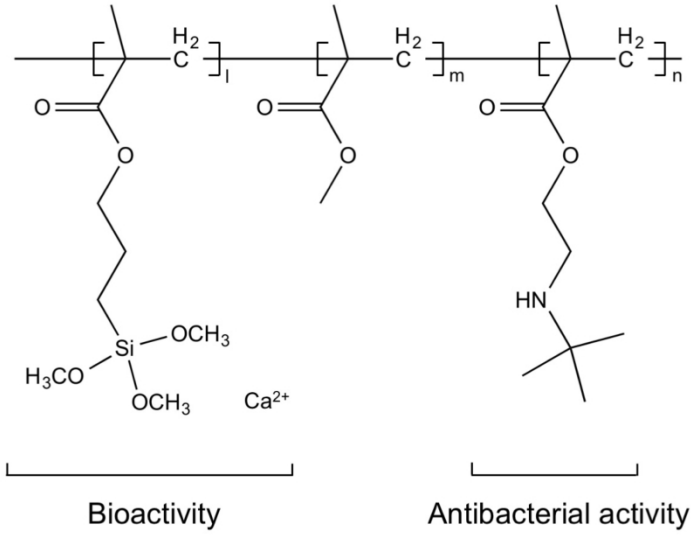

Fig. 1

Fig.1

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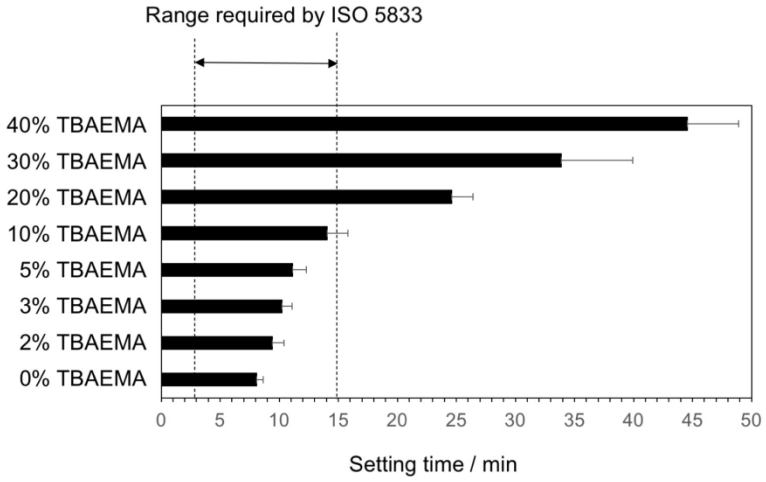

Fig. 2

Fig.2

225x145mm (600 x 600 DPI)

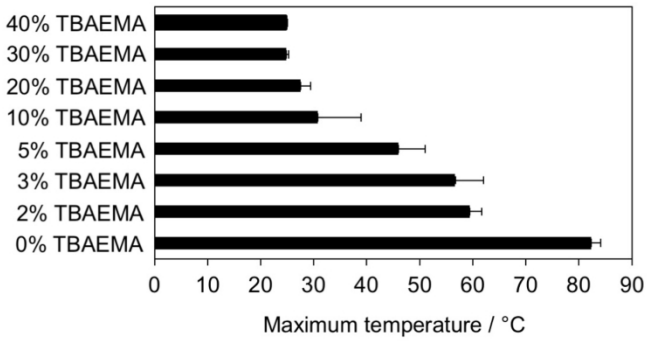

Fig. 3

Fig.3

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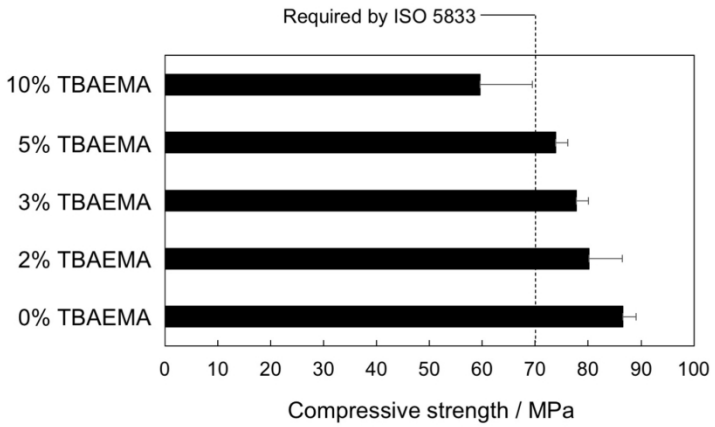

Fig. 4

Fig.4

215x133mm (300 x 300 DPI)

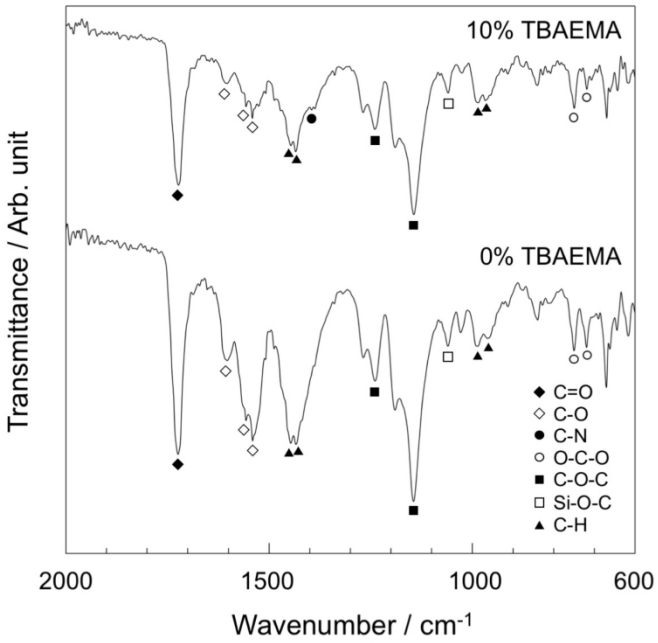

Fig. 5

Fig.5

201x154mm (300 x 300 DPI)

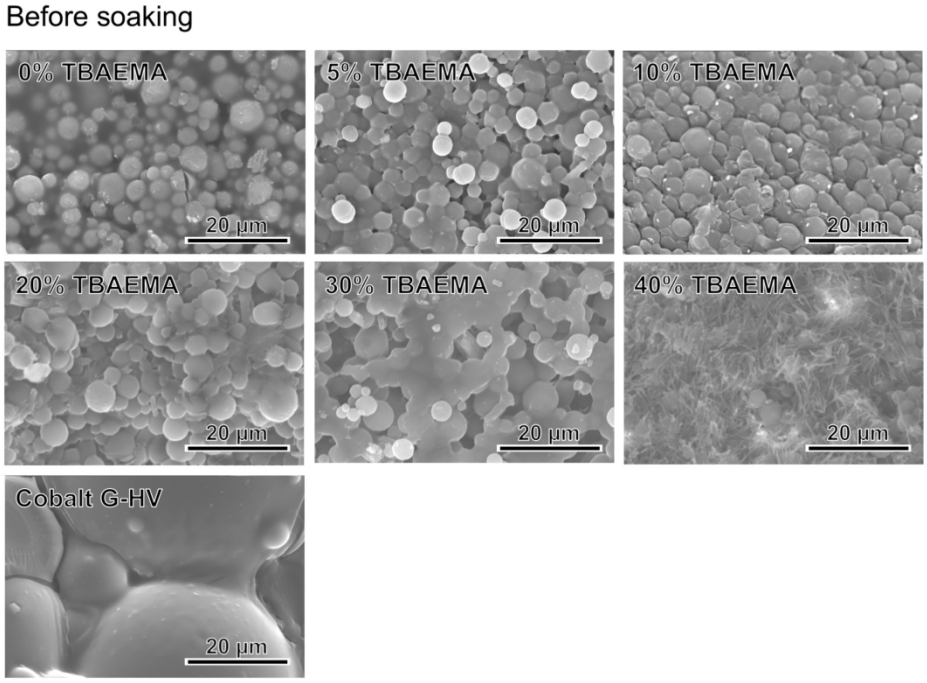

Fig. 6

Fig.6-1

225x170mm (300 x 300 DPI)

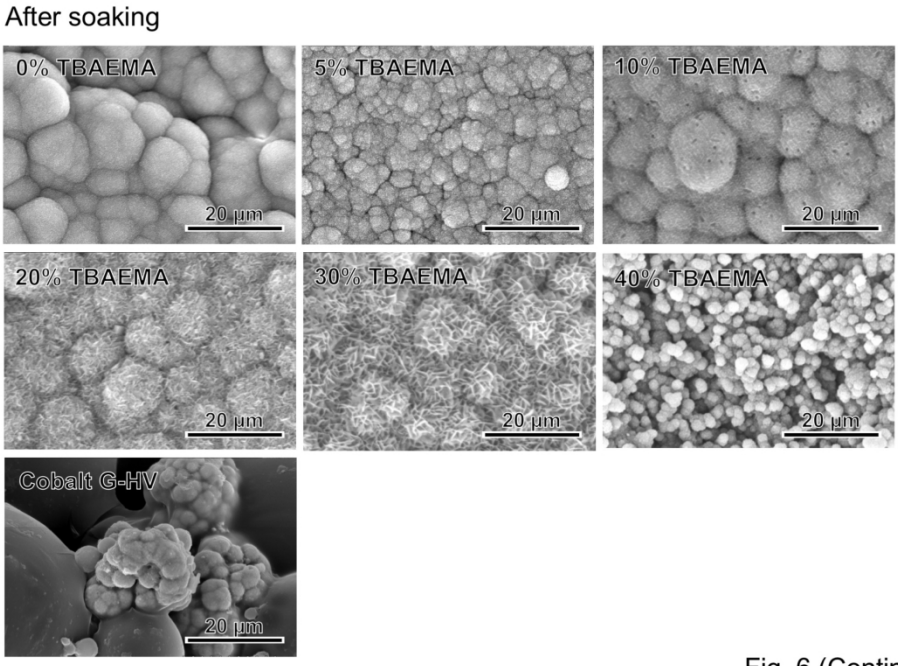

Fig. 6 (Continued)

Fig.6-2

238x171mm (300 x 300 DPI)

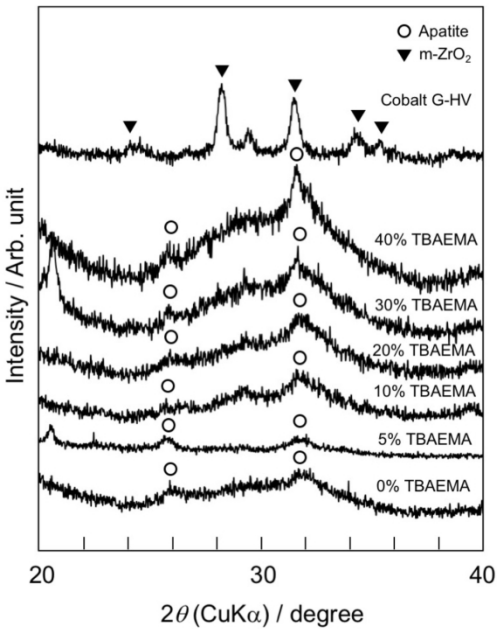

Fig. 7

Fig.7

176x147mm (300 x 300 DPI)

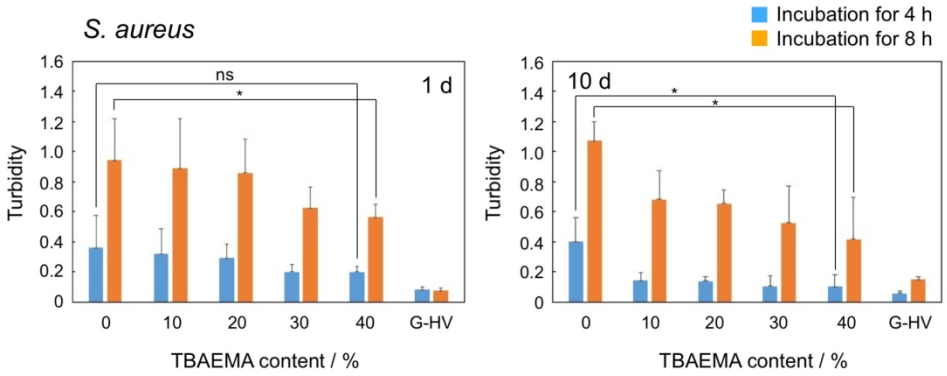

Fig. 8

Fig.8-1

243x129mm (300 x 300 DPI)

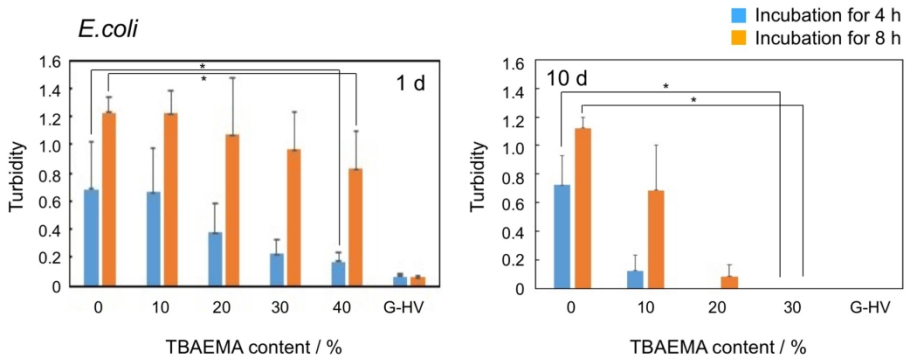

Fig. 8 (Continued)

Fig.8-2

258x139mm (300 x 300 DPI)

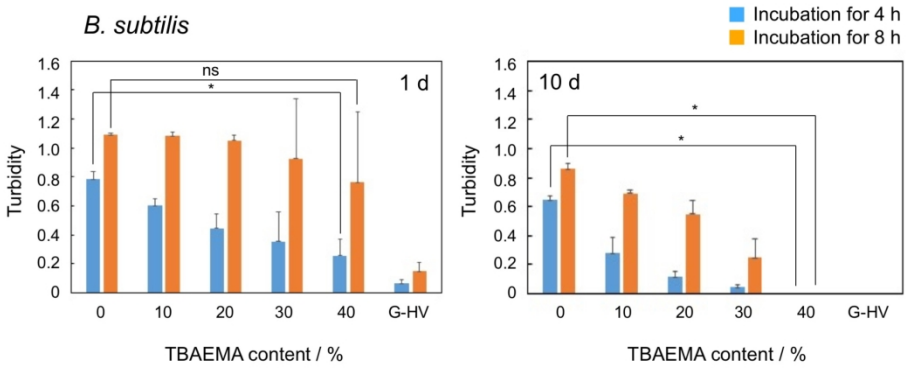

Fig. 8 (Continued)

Fig.8-3

256x135mm (300 x 300 DPI)

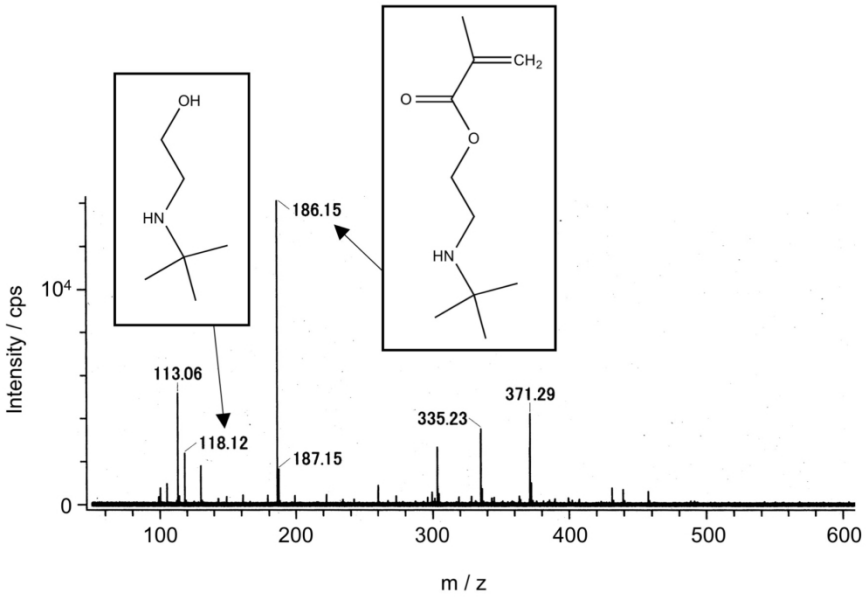

Fig. 9

Fig.9

232x167mm (300 x 300 DPI)