Is benzoyl peroxide detectable under physiological conditions in orthopaedic cement?

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Abstract: *Background*: Until today, benzoyl peroxide (BPO) as a potential polymethyl methacrylate (PMMA) cement allergen is the subject of controversial discussion. *Question/Purposes*: Few cases have been reported in literature and ought to be seen critically. To address this issue, the present

study aims to determine BPO and their degradation products biphenyl (BP) and benzoic acid (BA) within hardened cement as well as their elution from cement. *Methods*: Six ordinary cements characterised by different BPO: DmpT ratio were chosen for the study. Subsequently, cements were tested in-vitro under destructive, physiologic and non- physiologic conditions. *Results*: BPO was not detectable under destructive and physiological conditions. Cements with a N, N-Dimethyl-para-toluidine (DmpT) surplus contained lower BPO, BP and BA concentrations compared to cements with BPO surplus. *Conclusions*: Furthermore, the ratio of DmpT:BPO had an impact on the turnover of BPO and its degradation products. *Clinical Relevance*: Moreover, BPO and BP elution under physiological conditions was not detectable (<0.1 µg/mL in saline solution at a ratio of 1+9 cement to saline solution), calling into question whether BPO is relevant as PMMA cement allergen.

Keywords: PMMA; polymethyl methacrylate; BPO; benzoyl peroxide; allergy; in-vitro; HPLC; high performance liquid chromate-graphic system; low grade infection, COPAL PALACOS.

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1 Introduction

Polymethyl methacrylate (PMMA) bone cement is widely used in artificial joint replacement for fixation of total joint replacements. It buffers forces placed upon the joint. Moreover, it can be utilised as a carrier matrix for antibiotics and functions as a local drug-delivery system. Bone cements are a two-component system consisting of a liquid (or monomer) and a powder (or polymer) component.

The final PMMA cement is produced right in the operation theatre by mixing the components according to the manufacturer's instructions. The liquid component includes methyl methacrylate (MMA), N, N-Dimethyl-para-toluidine (DmpT) and hydroquinone (HQ). Depending on the brand, it may further contain colouring agents and/or other additives. The powder is composed of polymers and/or copolymers (PMMA and/or MMA), benzoyl peroxide (BPO) and sometimes colouring agents and antibiotics (Kühn et al., 2014).

The various ingredients of PMMA cements can potentially elicit allergic reactions that causing eczema, delayed wound or bone healing, recurrent effusion, pain or implant loosening (Carlsson et al., 1980; Deb, 2008; Goodman, 1996; Haddad et al., 1996; Rodgers et al., 1997; Hallab, 2001; Thomas et al., 2004). As a consequence, the body may respond with an inflammatory reaction that could lead to osteolysis and loss of the implant (Kubba et al., 1981; Thomas et al., 2004; Willert et al., 2005).

Especially BPO is known to bear an allergy risk (39). The cases reports are sparse and should be regarded critically, though (Pegum and Medhurst, 1971; Jäger and Balda, 1979; Romaguera et al., 1985; Haddad, 1995; ASTM Specification F 451-476, 1978; Meel, 2004; Richter-Hintz et al., 2005; Schuh et al., 2004; Thomas et al., 2004, 2006; Wetzel and Thomas, 2004; Schuh et al., 2006; Edwards and Gardiner, 2007; Gothner et al., 2011; Wawrzynski et al., 2017).

All cements on the market contain BPO which will rapid react as initiator with the activator DmpT for radical polymerisation. They chemically interact at room temperature producing free radicals initiating polymerisation process. During the quick redox reaction in PMMA cement, BPO reacts to a benzoyloxy radical and a benzoate anion, and DmpT to a radical cation (Nussbaum et al., 2004).

The quantities of BPO within the cement powder component from different brands may vary (0.5 to more than 2%), which essentially impacts temperature and cement dough setting. A higher amount of BPO will harden the dough faster while the temperature increases proportionally. Lower amounts of BPO will slow down hardening with proportionally lower temperature (Kruppke, 2010). The BPO: DmpT ratio should be considered, since a BPO surplus may favours a complete turnover of DmpT, while a DmpT surplus may lead to quantitative consumption of BPO. Theoretically PMMA cements with an activator surplus can significantly lower the risk of peroxide remaining in the hardened matrix. Because of its slight solubility under physiological conditions, BPO is supposed to remain in the bone cement matrix as well. Even if small amounts are released over time, the effect on the body is regarded as non-critical. BPO is rapidly metabolised to benzoic acid (BA) and CO2 (Ege, 1993; Shintani, 1993; Kühn, 2000). The induced cell damage is reversible in the absence of the chemical agent (AMG, 2004). Analytic tests are still needed to monitor the behaviour of BPO and its degradation products in cement moulds.

The present study aims to determine the BPO content in selected cement brands. Palacos® R, Copal® G + V and Antibiotic Simplex® with Tobramycin were chosen representing cements containing a DmpT surplus, while Hi Fatigue®, CMW® 1G and Cemex® Genta represent cements with a BPO surplus. BPO elution behaviour from various cement moulds were tested under destructive, physiologic and non- physiologic conditions in-vitro.

2 Materials/Methods

2.1 Cements

Six ordinary cements were selected. According to their composition, they were subdivided into two groups: Group 1 were cements containing a DmpT surplus (Palacos® R, Copal® G + V, Antibiotic Simplex® with Tobramycin). Group 2 were cements containing a BPO surplus (Hi Fatigue®, CMW® 1G) Cemex® Genta. The values of BPO and DmpT content given by the manufacturers are summarised for all cements (Table 1).

Table 1 Detailed BPO and DmpT content according to the manufacturer's indication for each cement

Cement	*P:L*	*BPO [%]*	*DmpT*	*Molar [%] ratio BPO:DmpT*
Palacos® R	2:1	0.75	2	1:2.4
Copal® G+V	2:1	0.75	2	1:2.4
Antibiotic Simplex® T	2:1	1.24	2.5	1:1.8
Hi Fatigue	2:1	0.84	0.65	1:0.7
CMW® 1G	2:1	1.95	≤1.50	1:0.7
Cemex® Genta	2:0.75	3.00	1.80	1:0.4

L: liquid; P: powder.

2.2 Producing mouldings

Powder was mixed with the liquid according to manufacturer specification' instructions. Curing took place in a silicon mould. PALACOS® R and Hi Fatigue® (representative cements) were performed as triplicate. The other cements were prepared once.

2.3 High performance liquid chromatography method (HLPC)

Samples were analysed with a high performance liquid chromate-graphic system (HPLC, 1260 Series, Agilent Technologies, Germany, Waldbronn) equipped with a variable wavelength detector for UV detection (adapted from Gaddipati et al., 1983). For the separation of the investigated compounds a Zorbax SB-Phenyl narrow-bore column (2.1 x 150 mm, 5 µm, Agilent Technologies, Germany, Waldbronn) (Romaguera et al., 1985) was used.

The column temperature was set to 40°C. Standards of five different concentrations (0.1, 0.5, 1, 10 and 100 µg/mL) of benzoic acid, biphenyl and BPO were used for calibration prior to the analysis.

2.4 Sample preparation

2.4.1 BPO content in powder components

An aliquot (~100 mg, weighed to 0.1 mg) of the respective PMMA powder was mixed with 10 mL acetonitrile. Subsequently, 1 mL of sample was mixed with 9 mL mobile phase, leading to the precipitation of the dissolved polymer. Each powder component was prepared three times.

2.4.2 BPO content in cement moulds

"Destructive" sample: An aliquot (~0.5 g, weighed to 0.1 mg) of the respective cement prepared as mentioned above was dissolved in 20 mL acetone over a period of 2 h. 'Physiological Condition' sample: One cement mould (~0.5 g, weighed to 0.1 mg) of the

respective cement was added to 5 mL NaCl (0.9% m/m) solution and mixed for 30 min. 'Non-physiological Condition' sample: One cement mould was added to 5 mL mobile phase and mixed for 30 min.

2.4.3 BP and BPO concentration in dissolved cement moulds

To determine the BP and BPO concentration under non physiological conditions is in cement moulds of the selected brands, ~0.5 g of each mould was dissolved in acetone for 2 h and analysed.

2.4.4 BA, BP and BPO concentrations in NaCl extracts

To mimic physiological conditions, cement moulds were incubated for 30 min in 5 mL NaCl.

The limit of quantification was 1 µg BPO/g cement.

2.4.5 BA, BP and BPO concentrations in mobile phase extracts

To determine BPO+ (BPO and degradation products) release under non- physiological conditions, cement moulds were mixed with 5 mL mobile phase (MP) and incubated for 30 min. Subsequently, the extract was analysed by HPLC (Stea et al., 1997).

2.4.6 BA, BP and BPO concentrations in mobile phase extract after 24 h

Cement moulds were mixed with 5 mL mobile phase (MP) and incubated for 24 h.

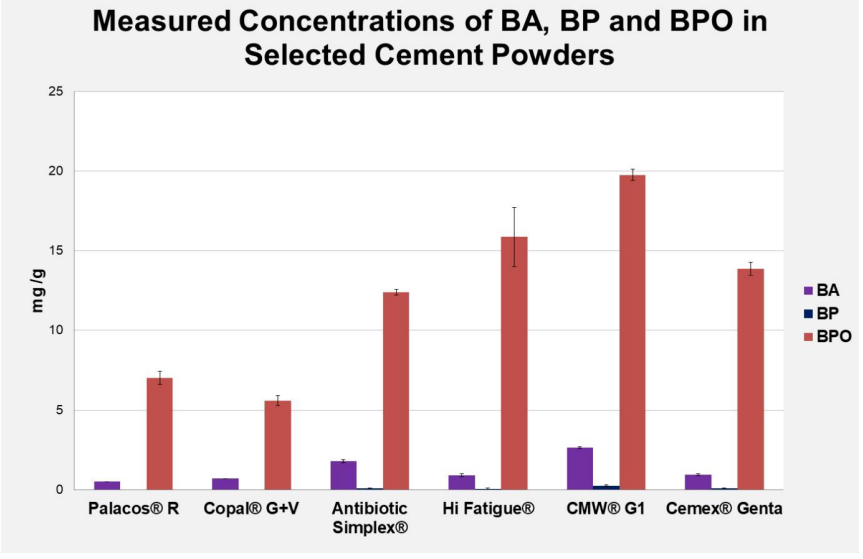
3 Results

The developed HPLC method with UV detection allowed the quantification of BA, BP and BPO in the polymer powder and the NaCl extracts. Furthermore, the quantification of BP and BPO was possible in the dissolved cement moulds as well as in the Mobile Phase (MP) extracts. Unfortunately, the BA quantification in cement moulds and MP were not possible as either the solvent (acetone) or other soluble cement constituents (colouring agents, antibiotics, etc.) interfered with the detection. The results were evaluated with the recoveries obtained from the spiking experiments of the determined compounds in the different experiments and provide reasonable statistical certainty.

3.1 BPO content in the powder of selected cements

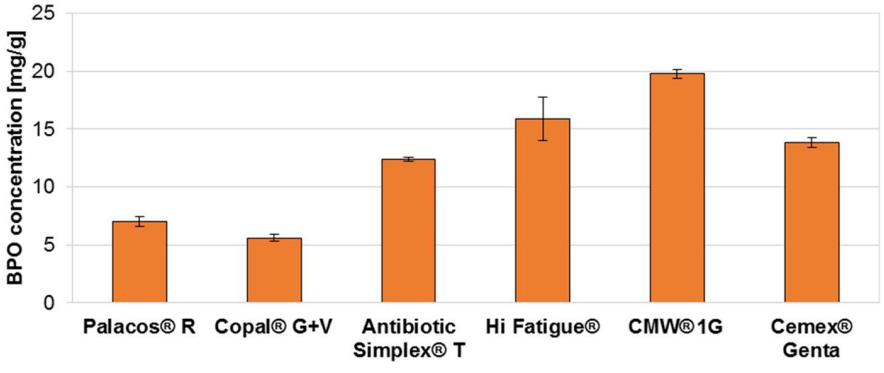
The detected BPO content was lowest in Copal® G + V (6 ± 0.3 mg/g) and PALACOS® R (7 ± 0.4 mg/g). Antibiotic Simplex® T (12 ± 0.2 mg/g), Cemex® Genta (14 ± 0.4 mg/g) and Hi Fatigue® (16 ± 2 mg/g) contained approximately twice as much. The highest BPO content was detected in CMW® 1G (20 ± 0.4 mg/g) (Figure 1).

Figure 1 Measured concentrations of BA, BP and BPO in selected cement powders (see online version for colours)



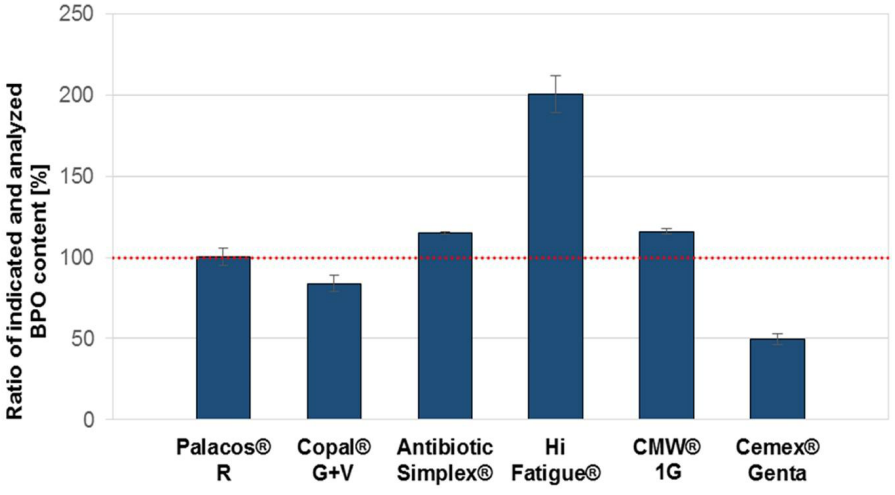
BP was absent in PALACOS® R (<0.05 mg/g) and Copal® G + V (<0.05 mg/g), while traces were detectable in all other cements. The lowest concentration was detected in Hi Fatigue® (0.1 ± 0.06 mg/g) followed by Antibiotic Simplex® T and Cemex® Genta with 0.1 ± 0.05 mg/g each, and CMW® 1G (0.2 ± 0.06 mg/g). (Figure 2).

Figure 2 BPO concentration in powder of selected cements (see online version for colours)



In 4 cases PALACOS® R (7.5 ± 0.4 mg/g), Copal® G + V (6 ± 0.3 mg/g), Antibiotic Simplex® T (14 ± 0.1 mg/g), CMW® 1G (23 ± 0.4 mg/g), the concentration measured in the cement powder was the same or close to the manufacturer's indications. The calculated BPO + concentration of the powder provided by Hi Fatigue® (17 ± 2 mg/g) was twice as high as expected concentration of 8.4 mg/g. The other outlier was Cemex® Genta, which contained only half the amount (15 ± 1 mg/g) of the expected BPO + concentration of 30 mg/g (Figure 3).

Figure 3 Relative distribution of determined BPO + concentration in comparison to the manufacturer's indications $n = 3$ (see online version for colours)



3.2 BP and BPO concentration in dissolved moulds

In general, the BP concentration was low in all samples analysed (Figure 4). In case of BPO, the concentration was low in PALACOS® R (500 ± 220 µg/g) and Copal® G + V (~470 µg/g) again. In all other samples, it was at least 14-fould higher with ~ µg/g detected in Antibiotic Simplex® T, 7100 ± 700 µg/g in Hi Fatigue® and 8800 µg/g in Cemex® Genta. The highest concentration was detected in the CMW® 1G sample, with ~14000 µg/g (Figure 5).

Figure 4 BP concentration in acetone dissolved cement (0,5g cement dissolved in 20 mL acetone; subjected to HPLC UV analysis after 2 h). Palacoa® R and Hi fatigue® $n = 3$, other cements $n = 1$ (see online version for colours)

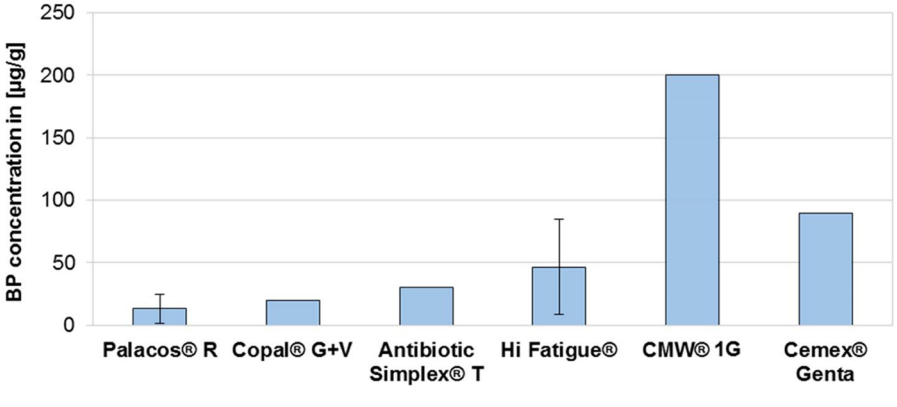
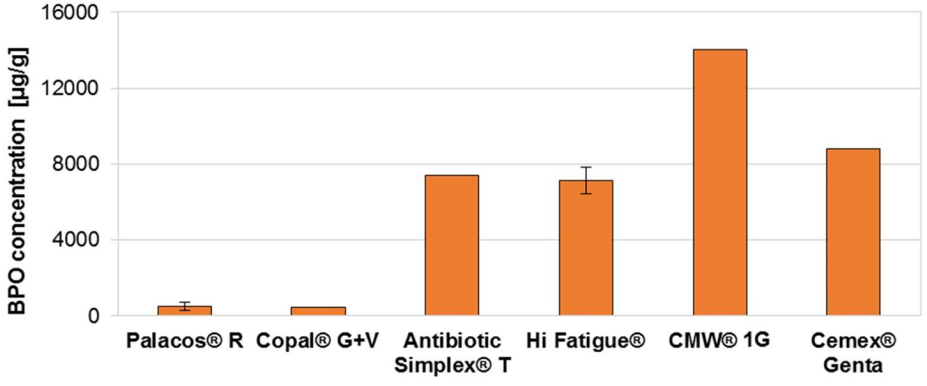


Figure 5 BPO concentrations in acetone dissolved cement (0.5 g cement dissolved in 20 mL acetone; subjected to HPLC UV analysis after 2 h). Palacos® R and Hi fatigue® $n = 3$, other cements $n = 1$ (see online version for colours)



3.3 BA, BP and BPO concentrations in NaCl extracts

In all samples, a low amount of BA was detectable (Figure 6). BP and BPO were not detectable in any sample analysed.

3.4 BA, BP and BPO concentrations in mobile phase extract after 24h

In case of BPO, the MP extract of the PALACOS® R and the Copal® G + V sample showed exceptionally low concentrations with 23 ± 8 µg/g and 8 µg/g, respectively. However, concentrations detected in the Antibiotic Simplex® T sample (~509 µg/g), Hi Fatigue® (707 ± 57 µg/g) and Cemex® Genta (~786 µg/g) where much higher, with a maximum concentration of ~1100 µg/g in CMW® 1G (Figure 7).

Figure 6 BA concentrations in NaCl extract (0.5 g cement in 5 mL naCl; subjected to HPLC UV analysis after 30 min). Palacos® R and Hi fatigue® $n = 3$, other cements $n = 1$ (see online version for colours)

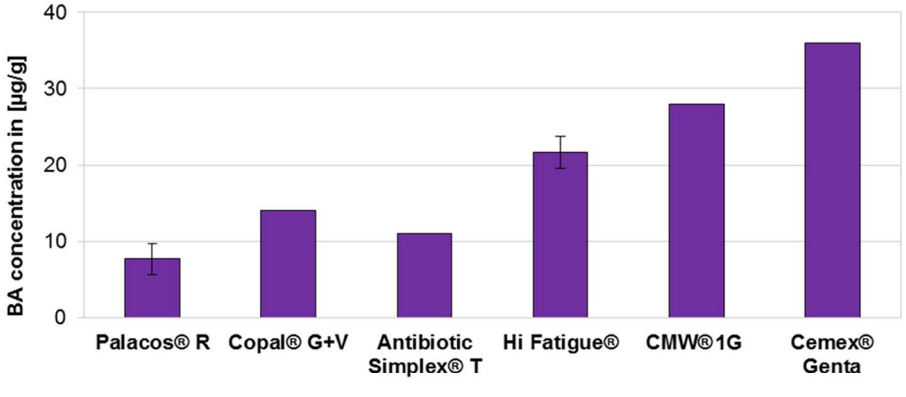
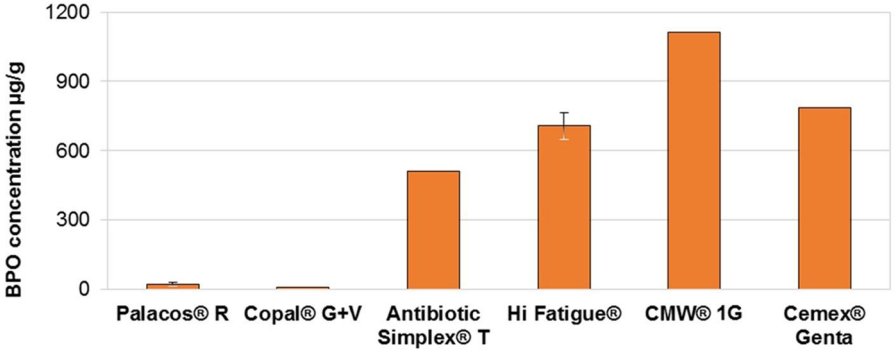


Figure 7 BPO concentrations in MP extract after 30 min. Palacos ® R and Hi fatigue ® $n = 3$, other cements $n = 1$ (see online version for colours)



3.5 BA, BP and BPO concentrations in mobile phase extracts

The DmpT surplus in Palacos® R did not allow the calculation of recovery of BPO.

In case of BPO, nothing could be detected in extracts of Palacos® R and Antibiotic Simplex® T (<1 µg/g cement). In addition, the concentration was extremely low in Copal® G + V (~3 µg/g). However, high concentrations were found in extracts of Cemex® Genta (~213 µg/g), Hi Fatigue® (~703 µg/g and 689 µg/g) and CMW® 1G (~1380 µg/g) (Figure 8). This shows clearly the influence of the DmpT surplus as all samples from group 1 show a decrease of the BPO content, whereas the cements of group 2 show constant BPO concentrations (Figure 9).

Compared to the measured BPO + content in the powder component of the different brands, most BPO + was detected after destroying the cement. Destructing the hardened cement mould by dissolving it in acetone. This represents the maximum present BPO after polymerisation. Palacos® R and Copal® G + V showed very low concentrations of BPO+ (without BA) compared to the other cements. The BPO + content (actually only BA, BP and BPO where not present) after 30 min incubation in NaCl was very low for all cements (8-36 µg/g) and very similar when compared to the initial BPO content (~0.1-0.3%), however slightly increased concentrations could be found in group 2 with BPO surplus.

Figure 8 BPO concentration in MP extracts after 24h. Palacos ® and Hi fatigue ® $n = 2$, other cements $n = 1$ (see online version for colours)

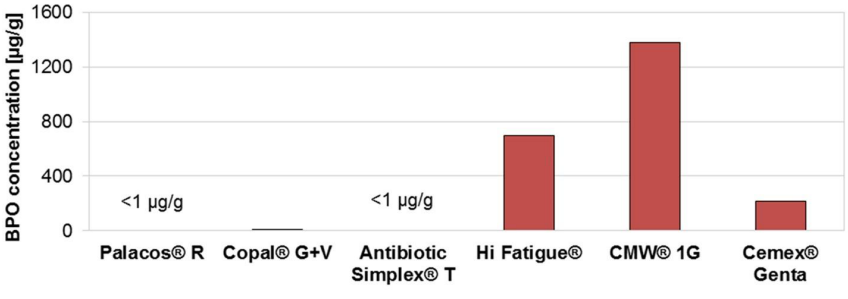
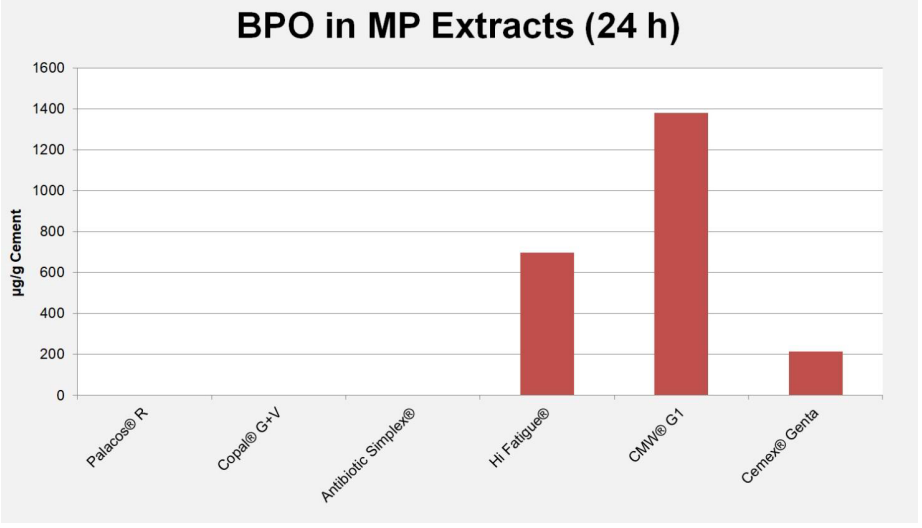


Figure 9 BPO concentrations in MP extracts after 24h. Palacos® R and Hi fatigue® $n = 2$, other cements $n = 1$ (see online version for colours)

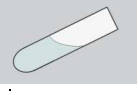


The 'Non-physiological Condition' samples show a significantly higher BPO + content in all cements, proofing the worst-case scenario. The BPO content in Palacos® R and Copal® G + V is very low though. The group 2 cements show higher BPO contents already after 30 min incubation and furthermore show still high concentrations of BPO after 24 h whereas the cements of group 1 with DmpT surplus show no remaining BPO anymore (BPO + content dominated by BP). However, the amount of BPO + varied between the cements under different conditions due to the difference of the input values. In cemented total joint arthroplasty, obviously more than 1 g cement is needed, and we estimated the total BPO concentration that could potentially be eluted from 40 g cement moulds under 'worst-case' conditions. The total BPO or BPO + content proportionally increases to the amount of utilised cement. Under non-physiological conditions after 30 min incubation in MP, which will not occur in the human body. the data clearly demonstrates that BPO elution is extremely low in case of Palacos® R (~1 mg/40 g) and Copal® G + V (~0.4 mg/40 g) compared to all other cements investigated (Antibiotic Simplex® T: ~21 mg/40 g; Hi Fatigue®: ~28 mg/40 g; GMW® G1: 45 mg/40 g; Cemex® Genta: 32 mg/40 g) and even under harsh conditions (Table 2).

4 Discussion

HPLC analysed the components of six selected cements characterised by different BPO:DmpT ratio (Palacos® R, Copal® G + V, Antibiotic Simplex® T, Hi Fatigue®, CMW® 1G a n d Cemex® Genta). BPO and small amounts of its degradation products BA and BP were detected. This raises the question how these degradation products interact with DmpT and if they potentially impact on the cement quality.

Table 2 Overview of the BPO + Content measured in the polymer powder and all compounded cements in µg/g and in percent [%] of the starting BPO + content in the powder considering the mixing ratio of powder and liquid cement components (indicated in brackets) (see online version for colours)

Cement brand	*Powder component*	*Hardened cement dissolved in acetone*	*Hardened cement after 30min in NaCl*	*Hardened cement after 30min MP*	*Hardened cement after 24h*
					
Palacos®R	7500 (100%)	500 (10%)	8 (0.2%)	24 (0.5%)	<1 (<0.02%)
Copal® G + V	6300 (100%)	490 (11%)	14 (0.3%)	9 (0.2%)	8 (0.2%)
Antibiotic Simplex®T	14300 (100%)	7430 (76%)	11 (0.1%)	518 (5%)	16 (0.2%)
Hi Fatigue®	16800 (100%)	7180 (63%)	21 (0.2%)	709 (6%)	696* (6%)
CMW® 1G	22600 (100%)	14200 (92%)	28 (0.2%)	1122 (7%)	1413 (9%)
Cemex® Genta	14900 (100%)	8890 (81%)	36 (0.2%)	796 (7%)	230 (2%)

*Average of two individual measurements.

Comparing the measured BPO + concentrations revealed same or close values as given by the manufacturers in four cases (Palacos® R, Copal® G + V, Antibiotic Simplex®, CMW® 1G). However, BPO + concentration calculated for Hi Fatigue® was twice as high as expected (8.4:17 ± 2 mg/g), while Cemex® Genta contained only half the amount of the expected BPO+ (30:15 ± 0.5 mg/g). Discrepancies between the values given by the manufacturers on the package and measured BPO content was already reported by Imai and Ohyama (2001). Remarkable is the good coincidence between measured concentrations and manufacturer declaration for products being established on the marked for a long time. Unlike Palacos® R, Copal® G + V, Antibiotic Simplex® T and CMW® 1G, Hi Fatigue® and Cemex® Genta were established later and under different legal regulations. Initially, all bone cement contents were defined as active substances by the pharmaceutical law. Thus, precise indication was obligate. However, nowadays and according to the Medical Device Guidelines, the manufacturer is not obligated to disclose the precise composition of the PMMA cement compounds (AMG, 2004), ASTM Specification F 451-476 (1978) and Kühn (2000). Consequently, the disclosures should rather be considered as being an indicative value that may vary between batches. Thus, precise content disclosures would be desirable, and manufacturers with extremely deviating values should reconsider their indications. To analyse the BPO and BP content in the hardened cement, moulds were prepared and dissolved in acetone.

A limitation of the experimental setting was that the analysis of BA was not possible, because acetone overlapped with the BA in HPLC chromatograms. As can be expected from the BPO/DmpT ratio, high BPO/BP concentrations were detected in Hi Fatigue®, CMW® 1G and Cemex® Genta, thus all cements that contained an initial BPO surplus compared to DmpT. A low BPO/BP concentration was detected only in Palacos® R and Copal® G + V, both cements with DmpT surplus compared to BPO. Indeed, a DmpT surplus seems to favour an increased consumption of BPO. However, Antibiotic

Simplex® with Tobramycin revealed high concentrations of BPO as well, although classified as Group 2 DmpT surplus cement. The reason may be that the ratio of BPO vs. DmpT is lower in Antibiotic Simplex® T (1:1.8) compared to Palacos® R and Copal® G + V (1:2.4 each). The lower amount of DmpT might be crucial and could be responsible for the decreased consumption of BPO. The experiment merely evidences that BPO and degradation products (BPO+) remains in higher concentrations in the hardened cement if an initial BPO surplus exists.

To mimic physiological conditions, cement moulds were incubated in NaCl for 30 min. The above described findings demonstrate that only traces of BPO + are eluted under physiological conditions. In contrast to the low solubility of BPO under physiological conditions, BA is indeed soluble. Of course, this approach cannot be used to make an assessment about the BPO + release of cements in the human body. However, the experiment shows that the risk of BPO + release under physiological conditions is rather low, at least in the short term. It remains to be determined whether firstly ex vivo retrieved bone cement samples might still contain BPO and secondly abrasive bone cement wear/particle generation gives an additional possibility of BPO release. To investigate if BPO + can be eluted at all from hardened cements without their destruction, cement moulds were incubated in MP for 0.5 and 24 h and extracts were analysed. Cements were bloated after the treatment. Cements of Group 2 with DmpT surplus revealed lower BP values after 0.5 and 24 h. Again, the values were lower for Palacos® R and Copal® G + V compared to Hi Fatigue®. Nevertheless, detected BP values in all samples of Group 2 were much lower compared to Group 1. The same results were achieved after analysis of BPO, apart from Hi Fatigue® sample that contained similar BPO concentrations as Group 1 cements after 0.5 h. These results repeatedly show that a DmpT surplus favours increased consumption of BPO.

Thus, under non-physiological conditions it is in principle possible to elute BPO + from cement moulds. However, these conditions would not occur in the human body and the above described concentrations of BPO + will not be eluted under physiological conditions.

In conclusion, hardened cements of group 1 with DmpT surplus contained less BPO + compared to group 2 with BPO surplus under all tested conditions. Furthermore, the ratio of DmpT:PBO had an impact on the turnover of BPO. In all cases, BPO and BP elution under physiological conditions was not detectable, calling into question whether BPO is relevant as PMMA cement allergen. Especially, since BPO is rapidly metabolised in the body (Treudler and Simon, 2007). Indeed, Thomas et al. (2013) found that patch testing performed in arthroplasty patients with complaints revealed a BPO contact allergy in 8% of cases. However, testing in symptom free arthroplasty patients revealed a BPO contact allergy in 6.7% of cases, raising further doubts of BPO being the main cause of the complaints. This observation may indicate that respective patients were sensitised during the period of cementing when exposed to BPO. However, there are few reported cases like those described by Bircher et al. (2012) with potential relevant BPO allergy. Furthermore, the Deutsche Kontaktallergie- Gruppe (DKG) [German Contact Allergy group] has issued that patch testing with BPO-preparation has a high risk of irritative reactions and thus results may be misinterpreted by non-experienced examiner as ‘false’ positive results, as well (Geier et al., 2008). However, despite such pitfalls in allergology diagnostics, we recommend allergen testing in complicated cemented orthopaedic implants. The opposite example is gentamicin-allergy in cemented arthroplasty, since

i such allergy can be clearly detected by patch test

ii there is in fact prolonged release of gentamicin from bone cement (Thomas, 2015).

Based on our findings, we recommend a cement with DmpT surplus in case of positive BPO allergy, unless cementless total joint arthroplasty is not possible. A limitation of this study is that only BPO has been investigated. It would be interesting to analyse the DmpT values of the same cements under the same conditions, to compare the outcome with the present data. An additional future approach would be analysis of 'ex-vivo' bone cement samples obtained during revision surgery.

Conflict of interest

All authors declare that they have no conflict of interest.

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