ORIGINAL ARTICLE

Cytotoxicity Test of Indonesian-made PMMA Acrylic Bone Cement on Fibroblasts and Osteoblasts

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ABSTRACT

Introduction: Polymethyl methacrylate (PMMA) bone cement is widely used due to its affordability, durability, and suitability for large defects. However, Indonesia lacks PMMA products designed specifically for cranio-maxillofacial use. Institut Teknologi Sepuluh Nopember (ITS) has developed the first national PMMA bone cement for this purpose, using DmpT and DmoT as its catalysts. This study assesses the biocompatibility of ITS's PMMA bone cement through cytotoxicity testing on fibroblast and osteoblast cells, and also the first to evaluate the cytotoxicity of DmoT-based PMMA bone cement, a cost-effective alternative to conventional DmpT-based products in Indonesia. **Method:** Cytotoxicity tests were conducted on BHK-21 fibroblast cells and hFOB osteoblast cells using MTT assay. The tests compared two types of ITS's PMMA bone cement (PMMA-DmpT and PMMA-DmoT) to OGM® bone cement, a commercially established product. **Results:** ITS's PMMA bone cement showed no toxicity to fibroblast or osteoblast cells, with cell viability levels similar to OGM® bone cement. Among the tested materials, OGM® bone cement had the highest cell viability, followed by PMMA-DmpT and PMMA-DmoT. **Conclusion:** PMMA bone cement developed by ITS using DmpT and DmoT catalysts is non-toxic to fibroblast and osteoblast cells.
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INTRODUCTION

Craniofacial defects result in alterations to the anatomy and morphology of the cranial vault and facial bones, often caused by trauma, infection-related tissue necrosis, or surgical interventions. Reconstruction of these defects necessitates balancing functional restoration with aesthetic improvement (1). Autologous bone grafting remains the gold standard for bone defect reconstruction; however, its use is frequently limited by patient concerns such as pain at the donor site, the creation of a secondary defect to harvest the graft, or insufficient bone availability. In such cases, bone substitutes offer an alternative solution.

Polymethyl methacrylate (PMMA) bone cement is a conventional bone substitute widely utilized in clinical practice. Despite its advantages, PMMA is bioinert, lacking the ability to actively stimulate osteoblast activity or promote new bone formation (2). This lack of osteoconductivity represents a significant limitation, as osteocytes cannot adequately colonize the PMMA surface, which is essential for stable osseointegration. Consequently, implants made of PMMA may experience poor integration with surrounding bone tissue, potentially leading to aseptic loosening over time (3). Nonetheless, PMMA bone cement remains a preferred choice for craniofacial reconstruction due to its cost-effectiveness, chemical inertness, ease of in situ shaping, and resistance to biodegradation (4). Additionally, its mechanical properties are comparable

to those of natural bone (5), enabling it to address large defects when autologous bone grafts are unavailable. These characteristics ensure that PMMA continues to be a viable option for craniomaxillofacial reconstruction in specific clinical scenarios.

Polymethyl methacrylate (PMMA) is an acrylic resin-based implant material composed of two components: a powder and a liquid, which are combined to initiate polymerization. In commercially available PMMA bone cement products, the liquid component primarily consists of methyl methacrylate (MMA), along with additives such as N,N-dimethyl-p-toluidine (DmpT), which acts as a catalyst to accelerate the polymerization process, and hydroquinone, which serves as a stabilizer to prevent premature curing of the monomer during storage. The powder component typically contains PMMA particles, benzoyl peroxide (BPO) as a polymerization initiator, and radiopaque agents such as zirconia (ZrO2) or barium sulfate (BaSO4) to enable radiographic visualization (6).

Despite its widespread use, some components of PMMA bone cement exhibit cytotoxicity, which can negatively affect fibroblasts and osteoblasts—key cells involved in bone healing. BPO, for example, has been linked to cytotoxic effects on osteoblasts due to the generation of free radicals during the polymerization process (7,8). Similarly, unpolymerized MMA monomer can elicit an inflammatory response, inhibiting fibroblast proliferation and tissue repair (8,9). DmpT has been reported to disrupt the cell replication cycle in human osteoblasts, impede the growth of human gingival fibroblasts, and cause cytotoxicity in rat polymorphonuclear leukocytes (10). This cytotoxicity can interfere with osseointegration, potentially leading to complications such as aseptic loosening of the implant. Nevertheless, acrylic bone cement remains a viable option for bone graft substitution, provided that its application minimizes cell necrosis and other adverse effects.

Currently, various acrylic bone cement products are available in Indonesia; however, none are specifically designed for craniomaxillofacial applications. The reliance on imported bone cement, particularly those intended for long bone reconstruction, poses significant challenges due to high costs and limited accessibility. These issues are particularly pronounced in the treatment of maxillofacial defects, where specialized products are required but unavailable locally. In response to this need, the Sepuluh Nopember Institute of Technology (Institut Teknologi Sepuluh Nopember, ITS) has initiated the development of the first nationally produced bone cement, marking a critical step toward addressing these limitations and supporting domestic healthcare advancements.

The catalyst commonly used in commercially available bone cement products is N,N-dimethyl-p-toluidine (DmpT). However, the limited availability of DmpT in Indonesia has prompted innovation by the Materials Engineering Department of the Sepuluh Nopember Institute of Technology (ITS). The department has explored the use of an alternative catalytic material, N,N-dimethyl-o-toluidine (DmoT), a toluidine derivative that differs from DmpT in the position of the methyl group on the aromatic ring. DmoT is more readily available in Indonesia and is also more cost-effective than DmpT. Acrylic bone cement cannot be immediately applied in the human body without thorough evaluation. Various tests are required to assess the body's response to the material, collectively referred to as biocompatibility testing. The specific tests required for acrylic bone cement are outlined in the International Organization for Standardization (ISO) 10993-1 (11). According to this standard, several biological evaluations must be conducted for materials intended as implants, namely physical and/or chemical information, cytotoxicity, irritation or intracutaneous reactivity, material mediated pyrogenicity, acute systemic toxicity, subacute toxicity, subchronic toxicity, chronic toxicity, implantation effects, genotoxicity, and carcinogenicity. These evaluations are needed to determine the suitability of acrylic bone cement material which will later be inserted as an implant into the body.

As for physical and/or chemical information, ITS has conducted preliminary testing of acrylic bone cement formulated with DmoT as a catalyst, adhering to standard procedures outlined in ASTM F451-21. These tests included Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), Fourier-transform Infrared Spectroscopy (FTIR), compressive strength tests, radiopacity evaluations, and antibacterial assays. The results demonstrated that incorporating DmoT as a catalyst influences the compressive strength and accelerates the polymerization reaction, reducing the setting time by 5–7 minutes and increasing the setting temperature of the bone cement by 6–9°C (12). When compared to DmpT, the setting time of PMMA-DmoT is longer, and it has lower temperature setting, as shown in Table I.

Table I: Comparison of physical and/or chemical information of ITS's PMMA bone cement

	Setting Time	Temperature Setting
PMMA-DmpT	7′	83°C
PMMA-DmoT	10′	77°C

Despite these promising findings, there is currently no research on the safety profile of DmoT in bone cement formulations. Further investigation is required to evaluate its biocompatibility and long-term effects. As for cytotoxicity evaluation, we performed in vitro test on fibroblast and osteoblast cells using the MTT assay. It is anticipated that the adoption of DmoT as a catalyst will enable the production of bone cement that is both safe and of comparable or superior quality to bone cement utilizing DmpT.

Finally, we will conduct an in vivo test using experimental animals to evaluate the rest of the biological evaluations (irritation or intracutaneous reactivity, material mediated pyrogenicity, acute systemic toxicity, subacute toxicity, subchronic toxicity, chronic toxicity, implantation effects, genotoxicity, and carcinogenicity).

In vitro testing in the form of cytotoxicity testing is carried out based on ISO 10993-5 (13), while in vivo testing is carried out on experimental animals based on ISO 10993-6 (14).

The goal of this research is to demonstrate that the PMMA bone cement developed by ITS Material Innovation is non-toxic to these cells, thereby establishing its safety for potential clinical applications.

MATERIALS AND METHODS

This study utilized an in vitro laboratory experimental design with a post-test randomized control group approach. Cell viability was assessed using the MTT assay on two cell cultures: baby hamster kidney fibroblast (BHK-21) cells and human fetal osteoblastic (hFOB) cells. BHK-21 fibroblast cells are placed on a culture plate for 5-10 minutes then the culture plate is added with MEM, 20% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 1% amphotericin B as a culture medium. hFOB osteoblast cells are placed on a culture plate for 5-10 minutes then the culture plate is added with DMEM, 20% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 1% amphotericin B as a culture medium. The culture plate is stored in a humid incubator (at 37oC, with 5% CO2) and the old media is replaced with fresh media twice a week. After the derived cells (which are spindle-shaped) migrate out of the tissue, the medium is changed to MEM and DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 1% amphotericin B, as the next culture medium. After the cells are released from the tissue, they are placed in a tube that has been added with trypsin EDTA. Then centrifuge for 5 minutes at a speed of 2.0 xg, then the supernatant is discarded and 1 ml of complete DMEM 10% medium is added. Then put into a flash and incubated in a 5% CO2 incubator. Every 4 days the medium is replaced with new medium. Observations are made under a microscope. When the cells are 80% confluent, the cells are divided into 96-well microplates.

The cultures were treated with acrylic bone cement formulations—PMMA-DmpT, PMMA-DmoT, and the commercially available OGM®1A bone cement—alongside a negative control group containing untreated cells. Treatments were applied for 24 hours, with each treatment was replicated twelve times per cell type. Cell viability was determined by measuring optical density (OD) using an ELISA reader at a wavelength of 540 nm. The mean OD values were used to calculate the percentage of cell viability according to the following formula (15,16):

% Cell Viability: Percentage of the number of living cells after testing
Sample OD : OD value in the well containing medium, cells (fibroblasts or osteoblasts), with treatment added (PMMA-DmpT, PMMA-DmoT, or OGM® bone cement)
Media Control OD : OD value in the well containing only medium
Cell Control OD : OD value in the well containing cells (fibroblasts or osteoblasts) without treatment (negative control)

A lower percentage of cell viability indicates a higher cytotoxic potential of the test sample. According to established criteria, a viability of less than 70% relative to the media control suggests that the sample exhibits cytotoxic potential (17). The MTT assay for this study was conducted at the Dental Research Center of Universitas Airlangga, Surabaya, in November 2024.

RESULTS

The results of the optical density (OD) measurements, average OD values, and cell viability percentages from the MTT assay using fibroblast cells are summarized in Table II. The findings indicate that none of the tested treatments—OGM® bone cement, PMMA-DmpT, and PMMA-DmoT—exhibited toxicity to fibroblast or osteoblast cells, as evidenced by cell viability percentages exceeding 70% in all groups. Among the treatments, OGM® bone cement showed cell viability percentages closest to the negative control, followed by PMMA-DmpT and PMMA-DmoT.

Table II. Optical density in the MTT assay with fibroblast and osteoblasts cells

		Media control	Cell control	PMMA-DmoT	PMMA-DmpT	OGM® *bone cement*
Fibroblasts	OD mean	0.092583	0.958417	0.879583	0.937417	0.946166667
	% Cell viability	0	100	90.89509	97.57459	98.58517806
Osteoblasts	OD mean	0.101833	1.320167	1.161083	1.236083	1.26675
	% Cell viability	0	100	86.94254	93.0985	95.61559508

OD: Optical density

To determine the appropriate statistical test for further analysis, the Shapiro-Wilk test was performed. All groups had p-values greater than 0.05, indicating that the data were normally distributed. Consequently, a one-way ANOVA was conducted, and the results are presented in Table III. In both fibroblast and osteoblast cells, p-values greater than 0.05 were observed, suggesting no statistically significant differences in OD among the three groups. Therefore, it can be concluded that the cytotoxicity of PMMA-DmpT and PMMA-DmoT is not significantly different from that of OGM® bone cement.

Table III. Analysis of optical density in the MTT assay with fibroblasts and osteoblast cells

	Groups	Mean	Std. Deviation	*p*
Fibroblasts	Cell control	.95842	.172289	.638
	PMMA-DmoT	.87958	.175447	
	PMMA-DmpT	.93742	.113877	
	OGM® *bone cement*	.94617	.172138	
	Total	.93040	.158295	
Osteoblasts	Cell control	1.32017	.354026	.498
	PMMA-DmoT	1.16108	.153975	
	PMMA-DmpT	1.23608	.246375	
	OGM® *bone cement*	1.26675	.231579	
	Total	1.24602	.254992	

DISCUSSION

The percentage of fibroblast cell viability for OGM® bone cement was the highest compared to PMMA-DmpT and PMMA-DmoT, with values of 98.6%, 97.6%, and 90.9%, respectively. A similar trend was observed in osteoblast cells, with viability percentages of 95.6%, 93.1%, and 86.9%, respectively. Although PMMA-DmpT demonstrated higher cell viability than PMMA-DmoT, the difference in optical density (OD) values between these two formulations was not statistically significant.

Toxic components, such as benzoyl peroxide (BPO), residual methyl methacrylate (MMA) monomer, and DmpT, are known to negatively impact fibroblasts and osteoblasts. However, this study does not assess the toxicity of each individual component but instead evaluates the solid PMMA bone cement as a fully polymerized product. This approach was chosen to simulate clinical scenarios where acrylic bone cement is mixed prior to use, either for in situ application or prefabrication. In such cases, the polymerization process predominantly occurs outside the body, meaning the material in contact with cells and tissues is already polymerized.

A limitation of this study is that it evaluates the fully solidified bone cement without simulating the in vivo continuation of the polymerization reaction during clinical application. Although mixing is performed outside the body, polymerization continues until the material hardens. This reaction can generate free radicals that are cytotoxic, as well as release heat from the exothermic reaction, potentially causing thermal necrosis. Additionally, residual MMA monomer from the polymerization process can elicit an inflammatory response, which may inhibit fibroblast proliferation and impede tissue healing. These aspects cannot be captured by this study and require further investigation through in vivo experiments using animal models.

The slightly higher cell viability observed in PMMA-DmpT compared to PMMA-DmoT may be attributed to the structural and chemical differences between the two catalysts. DmpT is the para-isomer, where the methyl group is positioned opposite the amine group on the aromatic ring. This configuration is less sterically hindered, allowing for more efficient electron donation during redox initiation with benzoyl peroxide (BPO). As a result, the polymerization process is generally more complete, potentially yielding fewer residual monomers and more stable polymer chains, which reduces cytotoxic effects on surrounding cells (6).

In contrast, DmoT is the ortho-isomer, and the proximity of the methyl group to the amine group introduces steric hindrance that can interfere with efficient free radical initiation. This may lead to incomplete polymerization and the presence of more residual MMA monomer or free radicals, which are known to have cytotoxic effects on fibroblasts and osteoblasts (2, 9).

Despite these differences, both formulations showed cell viability values above 70%, indicating that neither formulation is cytotoxic under the ISO 10993-5 criteria. However, understanding the polymerization efficiency and catalyst behavior is crucial for optimizing future

formulations.

CONCLUSIONS

In conclusion, PMMA bone cement developed by ITS using DmpT and DmoT catalysts has been demonstrated to be non-toxic to fibroblast and osteoblast cells in vitro. However, to comprehensively evaluate the safety of ITS's PMMA bone cement, further biocompatibility assessments, including in vivo testing on animal models, are recommended. Importantly, this is the first study to assess the cytotoxicity of DmoT-based PMMA bone cement, underscoring its potential as a more affordable and locally accessible alternative to DmpT-based formulations in Indonesia

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