

# *N*-Acetyl cysteine prevents suppression of oral fibroblast function on poly(methylmethacrylate) resin

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## Abstract

Despite the proven cytotoxicity, poly(methylmethacrylate) (PMMA) resin is one of the most frequently and extensively used materials in medical and dental fields. The study examined the potential detoxification of the resin material and restoration of the resin-induced suppression of cellular function using an antioxidant amino acid derivative, *N*-acetyl cysteine (NAC). Oral fibroblasts extracted from rat oral mucosa were cultured on the resin material with or without incorporation of NAC into the material. Twenty-four hour after incubation, less than 2% of the cells were viable on the untreated control resin, while up to 35% of the cells were viable on the resin with incorporation of NAC. At day 7 of culture, the expression of collagen I and III genes was downregulated on the untreated resin, while the cells on NAC-supplemented resin showed the expression levels similar to those in polystyrene culture. The cells produced three times greater amount of collagen on the NAC-supplemented resin than on the untreated resin. The data demonstrated that the cytotoxicity of PMMA resin was substantially lower when the material contains NAC. The potential usefulness of this principle should be explored with a view of developing biocompatible polymer-based materials in a broad range of dental and medical resin materials and tissue engineering scaffolds.

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## 1. Introduction

Resin-based materials are used for many applications in dental and medical fields. In addition to the physical and chemical properties, the clinical success of the materials depends also on their biological safety. Among these materials, acrylic-based self-polymerizing dental resin, which primarily consists of a solid part of prepolymerized poly(methylmethacrylate) (PMMA) and a liquid part of methyl methacrylate (MMA), is considered as one of the most frequently used resin materials in daily dental practice. It has been used successfully for the fabrication of temporary crowns, denture base and temporary seal of prepared cavities. However, its biological safety has been a

major concern [1]. MMA monomer is well known to cause allergic and/or toxic reactions [2]. Incomplete polymerization of PMMA dental resin under clinical conditions results in unreacted monomers that may be released from the resin matrix into the aqueous environment of oral cavity [3]. Such release of monomers is also observed even after complete polymerization of the resin [4]. Oral hypersensitivity reactions and local irritation caused by MMA and general toxicity and sensitivity to MMA have been reported [1,2]. Fibrosis, necrosis and histiocytosis are found locally in tissues around resin material [5,6].

At the cellular level, MMA monomer has been identified as cytotoxic by a variety of different methods, all indicating changes in basic cell structures, such as cell membrane integrity and cell functions like enzyme activities or the synthesis of macromolecules [7–9]. Although still not clear, the mechanisms of adverse effects caused by MMA monomer are thought to involve direct toxicity from released or

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residual MMA and oxidative stress created by free radicals that are released during the resin polymerization [10,11].

Fibroblasts constitute the predominant cell type of oral connective tissue, which contains both gingival and periodontal ligament fibroblasts. The preservation of integrity and health of these cells is essential for healthy related tissues. Studies utilizing permanent cell lines or primary culture of fibroblasts from various origins such as dental pulp, gingival and periodontal ligament demonstrated mutagenicity and cell death by exposure to MMA [7,9,12–14]. Therefore, the development of a biocompatible PMMA resin can help to overcome such adverse effects to fibroblasts.

The glutathione (GSH) is a cysteine derivative, and the glutathione mediated redox cycle is considered as the most important removal system for free radicals [15,16]. *N*-Acetyl cysteine (NAC) is a pro-GSH drug. It is easily deacetylated into L-cysteine, which is an important precursor of glutathione [17], and helps promote the cellular glutathione system [18,19]. It also acts as a direct strong oxidant scavenger [20]. It was therefore hypothesized that NAC has detoxifying and protective effects for fibroblasts against PMMA-based dental resin, which, if proven, might provide a novel solution to overcome the adverse effects of the PMMA-based resin.

The purpose of this study was to examine cell viability and function of oral fibroblasts when cultured on a PMMA-based auto-polymerizing resin material. Also, it was determined whether the impaired viability and function of the cells can be prevented on the material containing NAC.

## 2. Materials and methods

### 2.1. Resin preparation

Untreated control self-polymerizing dental resin (Uni-fast II, GC, Tokyo, Japan) was prepared by mixing the powder and liquid components (Table 1) for 15 s according to the manufacturer's recommendations (powder/liquid ratio of 0.6/0.4 g; total weight 1.0 g well<sup>-1</sup>) in a well (22 mm in diameter) of a 12-well cell culture-grade polystyrene dish. The resin mixture was spread over the well and the surface of the resin was leveled by vibrating the culture dish during its initial polymerization stage. The experimen-

tal NAC-supplemented resin was prepared by mixing the powder and liquid containing various concentrations of NAC (0.15%, 0.4% or 0.6% in weight percent of the final resin substrate). The concentration of NAC was determined by our previous study, in which PMMA-suppressed phenotypes of rat dental pulp cells were restored in the NAC-dose dependent manner [21]. The mixed resin was allowed to polymerize in the well at 37 °C for 30 min and rinsed with dd H<sub>2</sub>O once before seeding cells.

### 2.2. Oral fibroblast cell culture

Fibroblasts were obtained from palatal tissue of 8-week-old Sprague–Dawley rats. After sacrificing the animals, the palatal tissue was aseptically removed and washed with 1% phosphate buffered solution (PBS, MP Biomedicals, Solon, OH, USA). Then, the collected tissue was dissected into small pieces (<1 mm<sup>2</sup>) and digested with 0.25% collagenase for 12 h. The liberated cells were collected and plated in 100 mm plastic tissue culture dishes with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotic–antimycotic solution, containing 100 U ml<sup>-1</sup> Penicillin G sodium, 100 µg ml<sup>-1</sup> Streptomycin sulfate and 250 ng ml<sup>-1</sup> Amphotericin B in a humidified atmosphere of 95% air, 5% CO<sub>2</sub> at 37 °C. The cells were passed twice when they are at 80% confluency. After the second passage, the cells were detached using 0.25% Trypsin–1 mM EDTA-4Na and seeded directly onto either the cell culture-grade polystyrene dish, the untreated resin or NAC-added resin at a density of 2 × 10<sup>4</sup> cells cm<sup>-2</sup>. The medium was renewed every 3 days. This study protocol was approved by the University of California at Los Angeles Chancellor's Animal Research Committee.

### 2.3. Detection of cell viability and apoptosis

The viability and apoptosis of the oral fibroblasts was evaluated by flow cytometric detection of annexin V binding and propidium Iodide staining (Annexin V-FITC Kit, BD Bioscience, San Jose, CA, USA). This method is based on the binding properties of annexin V to phosphatidylserine (PS) and on the DNA-intercalating capabilities of propidium iodide (PI). The cells, incubated for 24 h on untreated resin or NAC-added resin, were tested, and the intensity of PI staining (*y*-axis) was plotted versus FITC intensity (*x*-axis). In all four plots, viable cells were seen in the left lower quadrant (3: annexin V – negative/PI-negative), early apoptotic cells in the right lower quadrant (4: annexin V – positive/PI-negative), late apoptotic/necrotic cells in the right upper quadrant (2: annexin V – positive/PI-positive), and necrotic cells in the left upper quadrant (1: annexin V – negative/PI-positive) [22–24].

### 2.4. Evaluation of cell density

At culture days 2 and 5, the cells were gently rinsed twice with PBS and treated with 0.1% collagenase in 300 µl of

Table 1  
Formulation of self-curing PMMA resin

Powder	Ethyl–methyl copolymer	70 wt.%
	Polymethylmethacrylate	30 wt.%
	CEBA	1.0 phr
	ACu	10 ppm
Liquid	Methylmethacrylate	95 wt.%
	Ethylene glycol dimethacrylate	5 wt.%
	LMAC	1.0 phr

CEBA, 1-cyclohexyl-5-ethyl barbituric acid; ACu, acetylacetone copper; LMAC, dilauryl dimethyl ammonium chloride.

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