

# Cytotoxic Effect of Composite Resin and Amalgam Filling Materials on Human Labial and Buccal Epithelium

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**Abstract:** Background and Objective: There has been a growing concern of the potential health hazards imposed by use of dental filling materials that include toxic compounds. So this study was designed to evaluate the percentage of apoptotic cells in the epithelium of buccal and labial mucosa after applying amalgam and composite filling materials. Materials and Methods: The epithelial cells were stained with fluorescence dyes; ethidium bromide, propidium iodide and monoclonal antiFas-1 antibody then examined under fluorescent microscope. Results: The cytotoxicity of amalgam was decreased with aging time while that of composite was increased. On the other hand, using antifas-1 antibody, it was found that the apoptotic cells were died through mitochondrial pathway. [Nature and Science 2010;8(10):48-53]. (ISSN: 1545-0740).

**Key words:** dental restorations, amalgam filling, composite resins cytotoxic effects.

## 1. Introduction

Oral and systemic cells are permanently exposed to various types of dental restorative materials, which may subsequently cause adverse effects where they can affect both the oral soft tissues adjacent to the restorations and give soft-tissue reactions at sites distant to the restorations [9, 32]. The most commonly used direct restorative materials are composite resins and silver/mercury amalgams [3]. Due to the variety of adverse effects of amalgam ingredients, especially the mercuric component, the controversial debate about the safety of amalgam fillings has continued to the present [29]. Resin composites are used to replace missing tooth structure and modify tooth color and contour, thus enhancing facial esthetics. Clinical studies have shown that composites are ideal for anterior restorations in which esthetics is essential and occlusal forces are low [5, 23, 28]. Since the polymerization reaction is never complete, methacrylic compounds in composite resins are released into the oral cavity tissues and biological fluids where they could cause local adverse effects [21]. Eluted (co)monomers and mercurials can reach concentrations, which might induce necrotic or apoptotic cell death in human gingival fibroblast (HGF) [24]. Therefore, the aim of the present work was to evaluate the percentage of apoptotic cells in the epithelium of buccal and labial mucosa after applying amalgam and composite filling materials.

## 2. Materials and Methods

Human oral mucosal samples were obtained

from the out clinic of Dentistry, Ain Shams University. The present work was conducted on 60 cases with amalgam dental restoration and 30 cases with composite dental restoration in vivo. The patients with age ranged from 20 to 35 years. The buccal samples from each patient with amalgam filling were taken after 15 min, 1 week and 3 years of filling insertion. Meanwhile, the labial samples from patients with composite filling were taken only after 15 min and 1 week of filling insertion. The mucosal samples from patients with amalgam filling were taken from both sides of inner cheek, the contact side (besides class I filling with buccal extension) and the opposite side as a control side. Meanwhile, the mucosal samples from patients with composite filling were taken from both lips, the contact upper lip (besides class V filling) and the lower lip as a control.

Oral mucosa samples collection:

- 1-The mouth was rinsed with normal saline to remove any exfoliated cells. Mucosal cells were then collected from each patient by gently scraping the cheek and the lip oral mucosa on the concerned areas with a sterile glass slide.
- 2- The cell suspensions were washed with the BPS (buffer phosphate saline) to separate the components of the saliva and centrifuged for 15 minutes at 1000 rpm.
- 3- The precipitated cells were collected for further investigation [1].

Fluorescence microscopic analysis of apoptosis:

#### 1- Ethidium bromide stain (EB):

Ethidium bromide used to identify apoptotic cells by staining the condensed chromatin. The epithelial cells were stained with 1  $\mu$ L EB for two hours then washed. The cells were examined under fluorescent microscope using low power (100X) and high power (200x & 400x) magnification. EB staining based on nuclear morphology (perinuclear chromatin condensation, nuclear collapse and eventual fragmentation). Calculation: For each sample, at least 100 cells were counted and the percentage of apoptotic cells was determined. Percentage of apoptotic cells = (total number of apoptotic cells / total number of cells counted) x 100 [2, 7, 11].

#### 2. Anti-human CD95 (Fas/Apo-1):

Monoclonal Ab against Fas/Apo-1 labeled by fluorescence was purchased from Sigma Company. 1/1000 dilution of stock solution was prepared as described by the agency. Buccal and labial cells were incubated with 10 $\mu$ L of anti CD95 as working solution for 30 min then examined under light and fluorescent microscope using low power (100X) magnification. Apoptotic cells that express CD95 will be labeled by green ring around the cell membrane. Calculation: For each sample, at least 100 cells were counted by the light microscope and the number of apoptotic cells labeled with Ab was counted by green microscope. Percentage of apoptotic cells = (total number of apoptotic cells / total number of cells counted) x 100 [4].

#### 3. Propidium iodide stain (PI):

Epithelial cells were resuspended in the Propidium iodide staining solution at dark room temperature for 5 min then washed. The stained cells were examined in a drop of propidium iodide stain using Fluorescent Microscope. Propidium iodide is a DNA-binding dye, stains dead cells that have lost their membrane integrity. Apoptotic cells appeared as spots of red and cells that are negative for PI were considered viable [8, 14, 17].

Statistical analysis:

One Way Anova (Post HOC LSD) was used for pairwise comparison between all groups [15 min, 1 week, and 3 years] of amalgam filling with all stains that were used. Student's t test was used for comparison between all groups [15 min, and 1 week] of composite filling with all stains that were used. Also, student's t test was used to compare between amalgam and composite fillings in groups, 15 min and 1 week with all stains that were used.

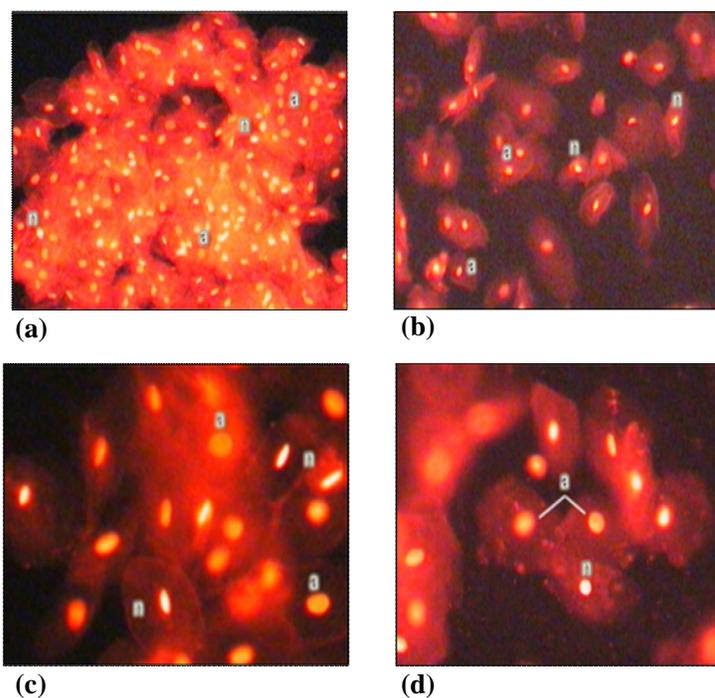
Significance of results:

Non significant	if P > 0.05
Significant	if P < 0.05
High significant	if P < 0.001

### 3. Results

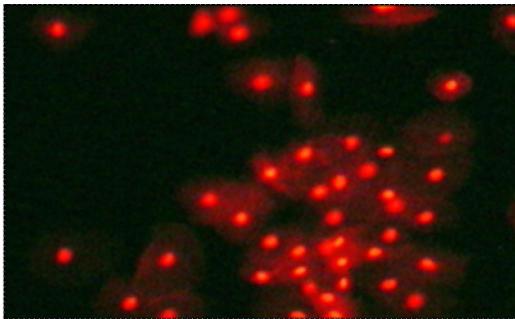
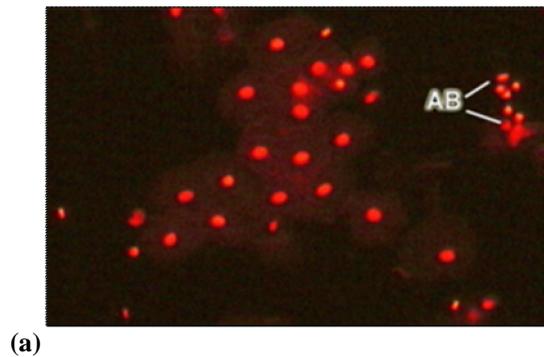
Using student's t test (2-tailed), there was a highly significant increase ( $p < 0.001$ ) in the number of apoptotic cells stained with EB and PI stains in the contact side when compared with the control side in each group of amalgam and composite fillings. The results also indicated that, the percentage of apoptotic cells in buccal epithelium caused by amalgam filling, decreased with increasing duration periods. It was 51.46%, 29.28% and 18.41% after 15 min, 1 week and 3 years respectively using EB stain.

Through PI stain, similar results were obtained. It was 48.29%, 30.24% and 20.56% after 15 min, 1 week and 3 years respectively. Meanwhile, the percentage of apoptotic cells in labial epithelium caused by composite-resin filling increased with increasing duration periods. It was 20.35% and 33.86% after 15 min and 1 week respectively with EB stain and it was 23.01% and 35.45% after 15 min and 1 week respectively with PI stain (Figures 1 & 2).



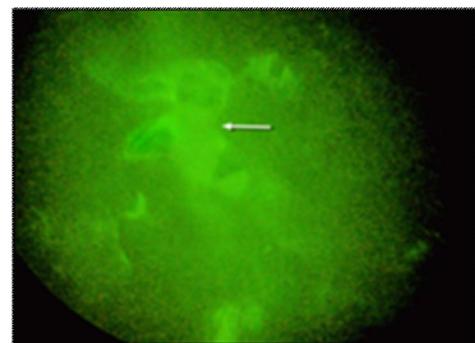
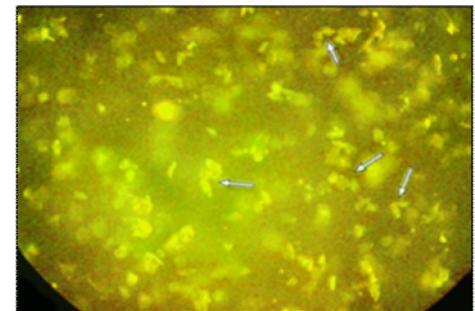
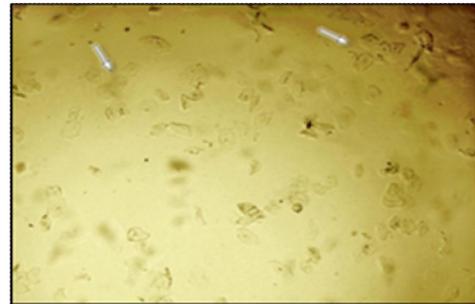
**Fig.1: Fluorescent microscopic pictures of buccal epithelial cells stained with ethidium bromide stain; (a) after 15 min, (b) after 1 week, (c) after 3 years of amalgam insertion;**

(d) with higher magnification to show apoptotic cells (a) with chromatin condensation and normal cells (n) with translucent nucleus.



**Fig.2:** Fluorescent microscopic pictures of labial epithelial cells stained with propidium iodide stain; (a) after 15 min, (b) after 1 week of composite insertion to show apoptotic cells with red nucleus and damaged cell membranes; some apoptotic cells seen in the termination phase that became apoptotic bodies (AB).

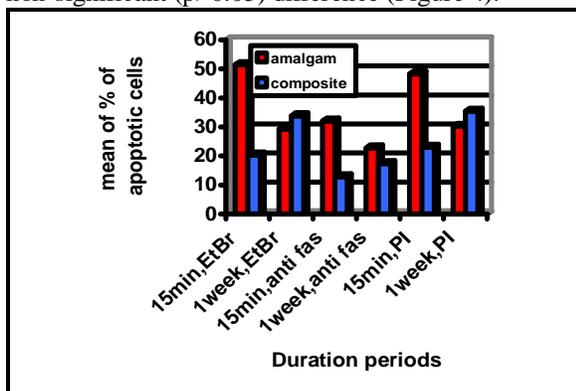
Also, a lower number of apoptotic cells labeled with anti-fas antibody was detected in both amalgam and composite fillings. Through anti-fas antibody, the percentage of apoptotic cells caused by amalgam filling was 32.03%, 22.76% and 16.49% after 15 min, 1 week and 3 years respectively. Meanwhile, the percentage of apoptotic cells caused by composite filling was 12.87% and 17.33% after 15 min and 1 week respectively (Figure 3).



**Fig.3:** Light (a & c) and fluorescent (b & d) microscopic pictures of epithelial cells labeled with anti CD95 antibody after 15 min of amalgam and composite insertion to show apoptotic cells with green cell membranes.

Using student's *t* test, there was a highly significant decrease ( $p < 0.001$ ) in the number of apoptotic cells in 15 min group of composite filling

when compared with that of amalgam filling with all stains. On the other hand, there was a significant increase ( $p < 0.05$ ) in the number of apoptotic cells in 1 week group of composite filling when compared with that of amalgam filling with EB stain while with PI stain and anti-fas antibody, there was non-significant ( $p > 0.05$ ) difference (Figure 4).



**Fig.4: Comparison between 15 min and 1 week groups of amalgam with 15 min and 1 week groups of composite.**

#### 4. Discussion

Decayed teeth can be restored by several dental restorations such as amalgam and composite fillings. The present work found that, there was a highly significant increase in the percentage of apoptotic cells in contact side when compared with control side due to a direct friction of mucosal cells to filling in contact side. Amalgam caused nearly 50% death to the buccal epithelial cells after 15 min of insertion and this can be explained as, dental silver amalgam releases up to 70%  $Hg^0$  vapor in the first day of filling insertion [15,19].  $Hg^0$  vapor more readily penetrates cellular membranes than inorganic mercuric salts because of its smaller size and high affinity for lipid membranes, so inhalation of  $Hg^0$  vapors results in greater Hg accumulation in all tissues [6, 15]. Furthermore, the percentage of apoptotic cells caused by amalgam filling was significantly decreased with increasing duration periods, and this is in agreement with Reichl *et al* [25]. The reduction in cytotoxicity with aging time may be explained as follows: (1) an oxidation of the amalgam surface retards the dissolution of elements from amalgam, and/or (2) the process of further amalgamation fixes residual mercury and other toxic elements in the amalgams [13]. On the other hand, cytotoxicity of dental composites has been firmly attributed to the release of residual monomers because of degradation processes or incomplete polymerization of the materials [33]. In contrast to amalgam, the present work revealed that the percentage of apoptotic cells caused by composite

filling was significantly increased with increasing duration periods, and this is in agreement with Samuelsen *et al* [27] and Schweikl *et al* [30]. It has been discussed that an increase of the periods of aging (extended up to 1week) will lead to an elevated rate of monomer conversion. In addition, it appears that the effect of aging might depend on the chemical nature of the various materials. Cytotoxicity of dimethacrylates such as TEGDMA and HEMA was increased after a long (72hr) exposure period indicating that treated cell cultures were unable to recreate from severe cell damage [30]. It has also been recognized that, composite resins are prone to enzymatic hydrolysis (by esterases) leading to the generation of toxic products such as methacrylic acid (MA) [20, 34], which can under certain conditions be oxidized to produce formaldehyde as a by-product [22]. Thus, resin biodegradation may play a significant role in producing changes in the oral environment [10, 34]. In addition the present work found that, there was a highly significant decrease in the number of apoptotic cells induced by composite filling after 15min of filling insertion when compared with amalgam while after 1 week of insertion, they had proximately the same cytotoxic effect. The higher toxicity of amalgam than composite through one day of insertion was observed by Kehe *et al* [16] and Reichl *et al* [24] who found a higher toxicity of mercury compounds when compared with the composite components after 24 hr of exposure. This may be explained by the interaction of mercury with critical proteins in the cells [16, 35]. An important reaction was the enzyme catalyzed reaction with reduced glutathione. After binding of metal ions (e.g., Hg) to glutathione, inorganic mercury can be detoxified, leading however, to a decrease of intracellular glutathione level. Furthermore, the cellular energy metabolism can be impaired rapidly and can lead to cell death [16]. On the other hand, a lower number of apoptotic cells labeled with monoclonal anti CD95 antibody indicates that, the cells were died through intrinsic pathway rather than extrinsic pathway and this is in agreement with the results of Guo *et al* [12], Lefeuvre *et al* [18] and Shenker *et al* [31]. In the extrinsic pathway, apoptosis is induced through surface receptors and activation of caspase 8, whereas in the intrinsic pathway, apoptosis is induced within the cells, mainly through permeabilization of mitochondria and activation of caspase 9 [26].

#### 5. Conclusion

The cytotoxicity is a primary factor of biocompatibility of dental restorative materials. Data from this study confirm that, a decreased toxicity was

found only for the amalgam filling between 15 min, 1 week and 3 years while for composite, the toxicity was increased between 15 min and 1 week. So the amalgam filling was preferred to restore the carious teeth especially in the posterior teeth due to its lowering toxicity by time and its high resistant to wear. Meanwhile, composite filling can be used to restore the anterior teeth only for aesthetic.

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