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Modified cytotoxicity evaluation of elastomeric impression materials while polymerizing with reduced exposure time

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Abstract

Objective. Cytotoxicity evaluation is an important step in biocompatibility assessment of dental impression materials. Previously, cytotoxicity evaluations were carried out on already set ('set') impression materials for contact time or extraction time of 24 h or longer in duration. However, clinically, dental impression materials are in contact with oral tissue while they are being set ('polymerizing'), for no longer than 10 min. Hence, the aim of this study was to investigate the difference in results between 'polymerizing' and 'set' forms of impression materials as well as the difference in results between longer duration of contact or extraction time (12 or 24 h) and shorter duration of time (15 or 30 min). ***Materials and methods.*** Seven dental impression materials of different chemical compositions were tested. Two commonly used *in vitro* tests were used—test on extraction and test by direct contact. Both 'polymerizing' and 'set' forms of impression materials were used with different durations of extraction and contact (15 min, 30 min, 12 h and 24 h). ***Results.*** There were significant ($p < 0.05$) differences of cell viability and cell proliferation between the 'polymerizing' and 'set' impression materials. Also, significant ($p < 0.05$) differences were noted with variance in duration of time. ***Conclusion.*** In light of the results, it is recommended to use a 'polymerizing' state of dental impression material for cytotoxicity evaluation, with 15 or 30 min of contact between cell and dental impression materials and an extraction time of 15 or 30 min that is more reflective of clinical situations.

Key Words: *biocompatibility, cytotoxicity evaluation, dental materials, elastomeric impression materials*

Introduction

Dental elastomeric impression materials have been used in dentistry for a long period of time to record the geometry of hard and soft dental tissues [1]. They have the advantages of elasticity, tear strength, rigidity, and reproduction of details, making them suitable to replicate intra-oral and extra-oral tissues [2,3].

The biocompatibility evaluation of dental impression materials is now considered an important step before their use on patients. The international standard for dental impression materials, ISO 4823 [4], does not contain specific information regarding biocompatibility evaluation of the material, but it recommends to refer to two other international standards, ISO 10993-1 and ISO 7405 [5,6]. Also, numerous studies were carried out to assess *in vitro* cytotoxicity of dental impression materials, which could be divided into two major tests; (1) direct contact test, where cytotoxicity evaluation was carried out following direct contact of impression materials with cells and (2) test on extract, where cytotoxicity evaluation was carried out following contact of cells with extracts of dental impression materials [6–12].

Many of the above tests were carried out using already set impression materials and cell cytotoxicity was evaluated following either 24 h or more duration of contact or 24 h or more duration of extractions [3,7,9,10]. However, clinically, the dental impression materials are in contact with oral tissues for no longer than 10 min while they are being polymerized ('polymerizing') rather than being already set ('set') [7].

Hence, in this experiment, tests were carried out on both 'polymerizing' and 'set' impression materials and the results were compared. Also, tests were

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carried out with different modes of time, including 15 and 30 min to compare the results with those of 12 or 24 h of contact between cells and dental impression materials and extraction of dental impression materials.

Materials and methods

Materials

Seven dental impression materials (one polysulfide, one condensation silicone, four vinyl polysiloxane and one polyether) were selected to make a complete spectrum of all types of dental impression materials for cytotoxicity evaluation. These are listed in Table I with their assigned code for the purpose of this paper. The 'set' form of impression materials was prepared by mixing and setting the impression materials according to the manufacturers' instructions and cutting the materials into a circular shape with 1 mm of diameter and 3 mm of height. The 'polymerizing' form of impression materials were prepared by directly applying equal weight of mixed impression materials on the test media using laboratory scale (AND, Japan) where the best effort was carried out to produce same sized and shaped samples to the 'set' form of impression materials by an experienced user. The weight of the 'polymerizing' impression material was determined according to the average weight of each 'set' impression material.

Test on extract

The test on extract was performed according to international standards [12,13] and other studies [3,7,8,10,11] with some modifications.

The extract of each impression materials was prepared in each well of standard 24 well plates (SPL, Korea), containing 2 mL of RPMI-1640 (Sigma, Irvine, Ayrshire, UK). The volume of the media was decided according to the international standard of ISO 10993-12 [13].

Extractions were carried out by incubating each of the samples for 15 min, 30 min, 12 h or 24 h at 37°C. For each of the time periods, impression materials were removed, and the extracts were kept inside the incubator until the removal of the last impression material at 24 h.

L929 cells (NCTC clone 929, Korean Cell Line Bank, KCLB, Korea) were plated at 1×10^4 cells per well in standard 96 well plates (SPL, Korea) in 100 μL of culture medium and were incubated at 37°C. Following the cell cultures reaching the sub-confluence, the medium was removed and the monolayer was washed with Dulbecco's phosphate buffer saline (DPBS, Welgene, Korea) and exposed to 100 μL of extracts and the fresh medium as the control for 24 h. Each condition was tested in quadruple wells and cell viability was estimated using water-soluble tetrazolium (WST) assay where values were compared relative to the control.

Test by direct contact

The test by direct contact was carried out according to international standards [12] and other studies [3,8–11] with some modifications.

Each of the 'set' impression materials and 'polymerizing' impression materials were placed in each well of standard 24 well plates (SPL, Korea) containing 2 ml of culture medium with fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) and antibiotics (100 IU/mL of penicillin and 100 μg/mL of streptomycin, Gibco).

L929 cells were plated at 5×10^4 cells per well immediately after placing each impression materials. The control was prepared by placing cells in the well containing only culture medium without impression materials. Each of the test and control groups was evaluated in quadruple wells.

Impression materials were removed after 15 min, 30 min, 12 h and 24 h. Cell proliferation was assessed using WST assay at 24 h by comparing values with the control.

WST assay

WST salt (Ez-Cytox, Daeillab, Korea) is a yellow water-soluble dye which is reduced by products of live cells to form water-soluble orange formazan dye.

Table I. Summary of materials.

Name	Code	Type	Batch no. and type	Manufacturer
Permlastic	PL	Polysulfide	0–1145 Type 3	Kerr, USA
Xantopren Comfort	XC	Condensation silicone	340330 Type 3	Heraeus, Germany
Imprint 3	I3	Vinyl polysiloxane	421057 Type 3	3M ESPE, Germany
Aquasil Ultra XLV	AU	Vinyl polysiloxane	101104 Type 3	Dentsply, Germany
Extrude Wash	EW	Vinyl polysiloxane	0–1328 Type 3	Kerr, USA
Delikit	DK	Vinyl polysiloxane	Od07759 Type 3	Happyden, Korea
Permadyne	PD	Polyether	418628 Type 3	3M EPSE, Germany

The amount of formazan generated is known to be directly proportional to the number of viable cells [14].

The volume of 10 μL and 200 μL of WST solutions were added for test on extract and test by direct contact, respectively. The absorbance was measured in a spectrophotometer at a wavelength of 450 nm after 3 h of adding WST solutions. The results were expressed as a percentage to the values obtained from the control for both experiments.

Statistics

The statistical analysis was carried out by one-way ANOVA and independent *t*-test (equal variance not assumed). The significance was declared at the $p < 0.05$ level. The SPSS PASW 18.0 program (SPSS Inc., Chicago, IL) was used for this statistical analysis.

Results

Test on extract

The results of cell viability following exposure to extracts of both 'polymerizing' and 'set' dental impression materials is shown in Figure 1. Statistically lower ($p < 0.05$) cell viability was observed on cells exposed to 24 h of extraction compared to when they were exposed to shorter durations of extraction for both 'polymerizing' and 'set' forms of PL, XC, and DK. Also, cell viability results for 24 h extraction of 'set' AU and PD, and 'polymerizing' I3, showed statistically lower ($p < 0.05$) cell viability compared to cells exposed to a shorter duration of extraction for each of the test groups.

When considering two different forms of dental impression materials, 'set' and 'polymerizing', there was a statistically significant ($p < 0.05$) difference in cell viability results between two forms in the test group of PL (24 h), XC (15 min), I3 (15 min, 12 h, 24 h), AU (15 min), EW (15 min, 30 min, 24 h), DK (12 h) and PD (15 min, 30 min), which have all shown lower cell viability for the 'polymerizing' group.

Test by direct contact

The result of cell proliferation following direct exposure to both 'polymerizing' and 'set' dental impression materials is shown in Figure 2. Statistically lower ($p < 0.05$) cell proliferation was observed on cells exposed to 24 h of extraction compared to those exposed to shorter durations of extraction for both 'polymerizing' and 'set' forms of PL, DK and PD. Also, there was a statistically significant ($p < 0.05$) difference in cell proliferation results between 'set' and 'polymerizing' forms of PL (12 h), although the result showed lower cell proliferation for the 'set' form, in contrast to the test on the extract.

Discussion

The dental impression materials are now considered as medical devices, which require a series of assessments for evaluation of their use on patients.

Along with physical and mechanical assessment, the biocompatibility evaluations of dental or medical materials are now considered as an essential step towards the acceptance of the materials, and the clinical relevance of such biocompatibility evaluation is now widely recognized, with increasing interest of an *in vitro* test as an alternative to the animal, *in vivo* test in the biological evaluation of dental materials [5,15–17].

The cytotoxicity assessment is a fundamental step in the evaluation of their biocompatibility [7,10] and in this experiment two widely used *in vitro* tests were carried out to assess cytotoxicity of dental impression materials—test on extracts and test by direct contact.

The results of these experiments were similar to previous experiments which showed relatively low levels of cytotoxicity for polysulfide [8] and high levels of cytotoxicity for polyether [10] and vinyl polysiloxane [8,9]. However, despite relatively low levels of cytotoxicity for condensation silicones in this experiment, other studies have shown high levels of toxicity with other condensation silicones [7]. Also, some studies showed no significant reduction in cell viability for polyether [9].

Many previous studies have suggested that a possible reason for such cytotoxicity is due to the by-product of polymerization, such as ethyl or methyl alcohol released during the polymerization of condensation silicones [7,9]. Also some of the components of materials such as the initiator, the accelerator, the surfactant or the metal ions, are known to be potentially cytotoxic, where one study showed cytotoxic effect on cells such as L929 and gingival fibroblast, by platinum and palladium present on vinyl polysiloxane [18].

It is well known that different results of the cytotoxicity test could be obtained with different cell types used in those experiments [11], and some suggested the use of human cells such as human osteoblast-like cells [8] or human gingival fibroblast cells [7]. The mouse fibroblast of L929 cells were used in this experiment, although use of oral epithelial cells might have been a better model.

The purpose of this experiment was not to identify or 'rank' cytotoxic dental impression materials but to find out differences in results according to duration of contact or extraction time and according to the state of samples such as either 'polymerizing' or 'set'. The results showed that there were significant ($p < 0.05$) differences in cytotoxicity between 24 h of contact or extraction, that were being previously tested to other periods of contact or extraction and even as short as 15 or 30 min in some test groups. Also, a significant

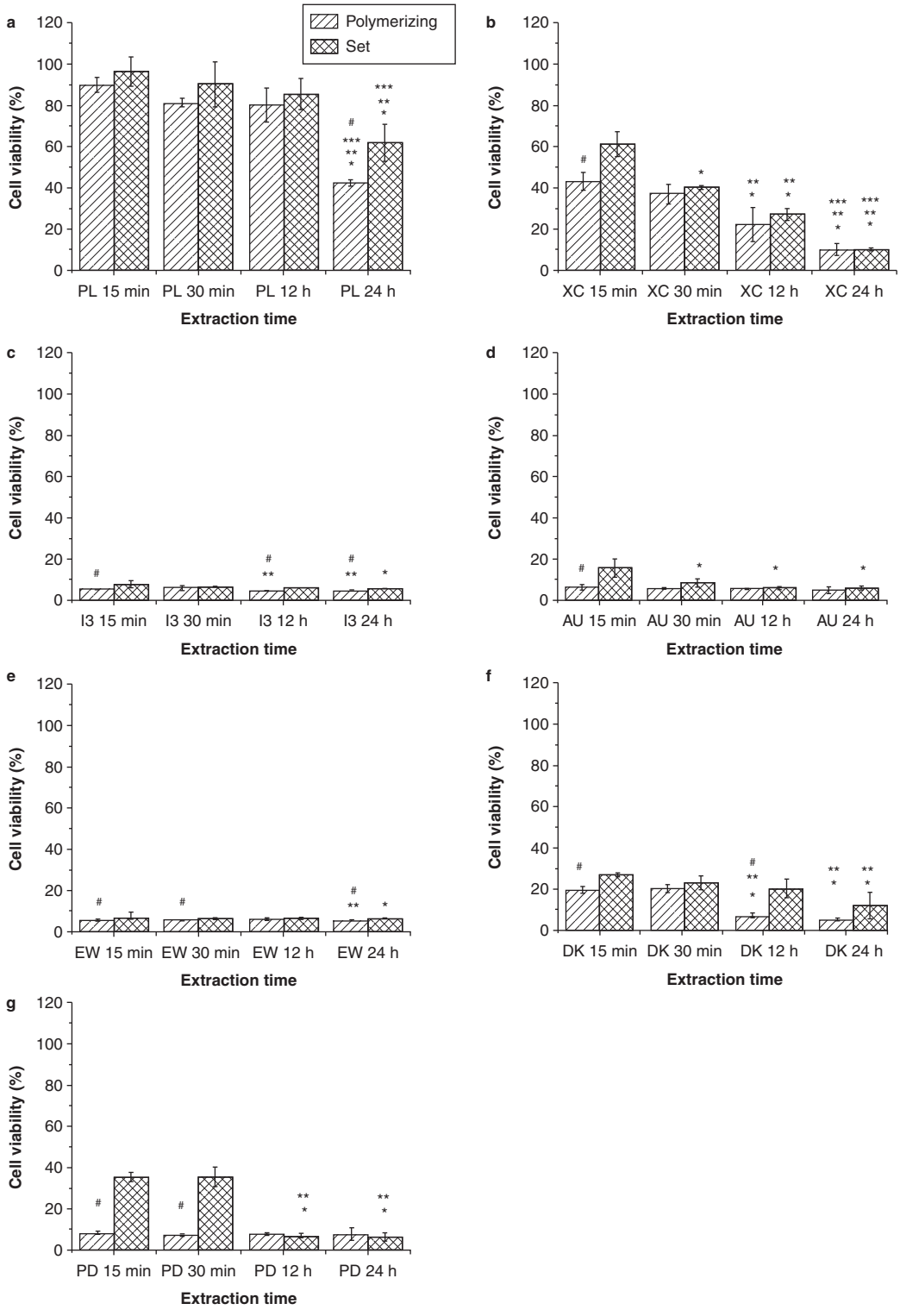

Figure 1. Cell viability following exposure to extracts of 'polymerizing' and 'set' impression material with different extraction time. (A) Permlastic, PL, (B) Xantopren Comfort, XC, (C) Imprint 3, I3, (D) Aquasil Ultra XLV, AU, (E) Extrude Wash, EW, (F) Delikit, DK, (G) Permadyne, PD. Significantly low ($p < 0.05$) cell viability compared to *15 min sample, **30 min sample, ***12 h sample, # 'set' or 'polymerizing' sample of same time period.

($p < 0.05$) difference was noted between 'polymerizing' impression material and 'set' impression materials in a few of the test groups.

The idea of this experiment began with clinical relevance of the *in vitro* experiment for dental impression materials where, clinically, dental impression materials are freshly mixed and in contact with oral tissue while they are being polymerized for no longer than 10 min [7]. Despite their extensive use, there were very few reports regarding adverse reaction

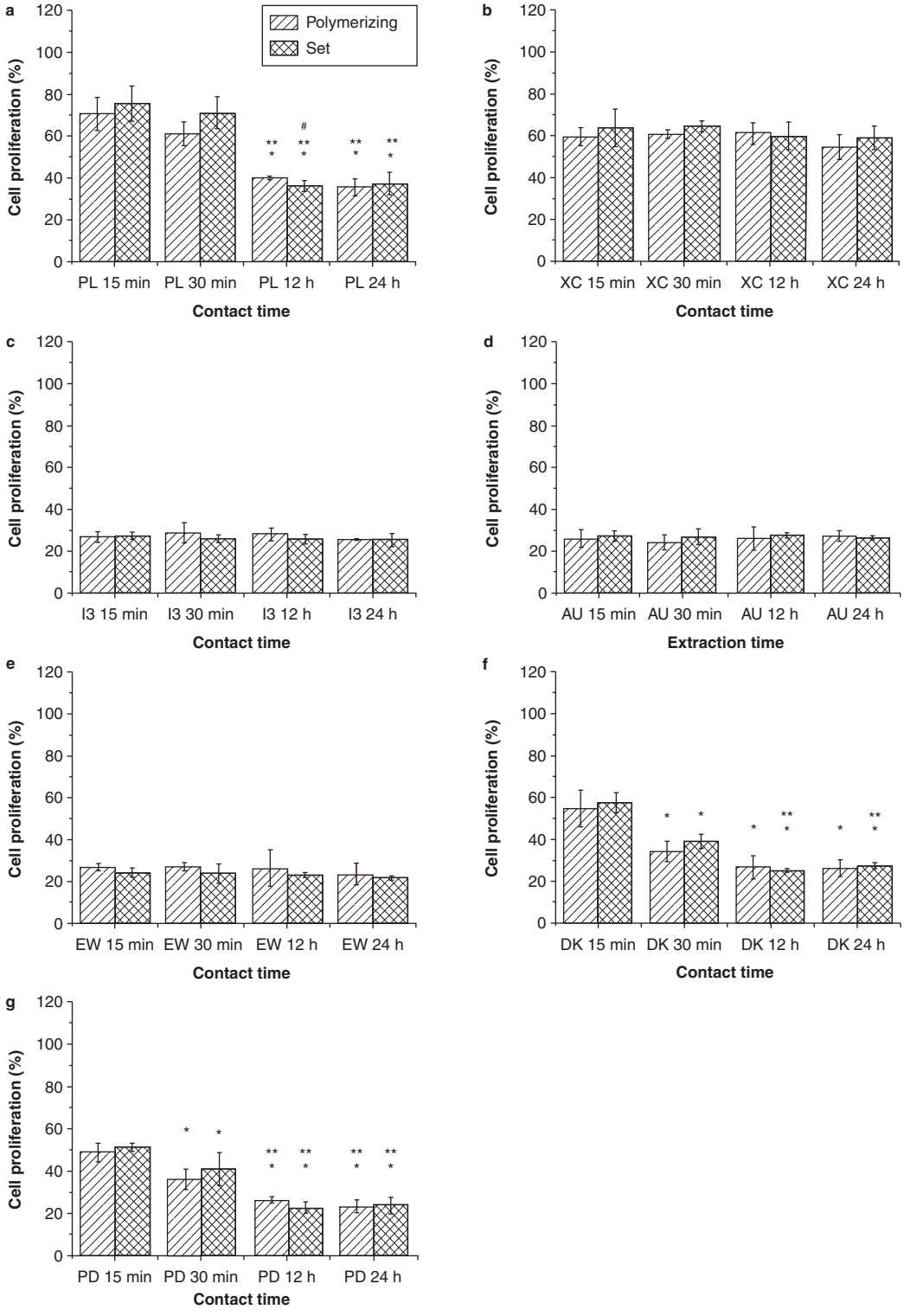

Figure 2. Cell proliferation following direct contact with 'polymerizing' and 'set' impression material with different contact time. (A) Permlastic, PL, (B) Xantopren Comfort, XC, (C) Imprint 3, I3, (D) Aquasil Ultra XLV, AU, (E) Extrude Wash, EW, (F) Delikit, DK, (G) Permadyne, PD. Significantly low ($p < 0.05$) cell viability compared to *15 min sample, **30 min sample, # 'set' or 'polymerizing' sample of same time period.

to dental impression materials by patients, unless pieces of materials were left behind in the oral tissue for a long period of time [8]. Also, criticisms had been raised with regard to poor correlation between some results of *in vitro* tests with *in vivo* tests [19]. Hence, in this experiment, *in vitro* tests were carried out to reflect clinical pictures and perhaps increase correlations with *in vivo* tests, although further *in vivo* tests would be warranted to examine this hypothesis. The results of this experiment showed that carrying out the

experiment according to clinical situations would be different to existing test methods, and this would be appropriate for future tests of dental impression materials, as they reflect an 'as-used' state of dental impression materials [6].

In light of the results, it is recommended to use a 'polymerizing' state of dental impression material for cytotoxicity evaluation, with 15 or 30 min of contact between cell and dental impression materials and extraction times of 15 or 30 min, which is more reflective of clinical situations.

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