

# Cytotoxicity of two self-adhesive flowable composites on bovine dental pulp-derived cells

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## ABSTRACT

**Aim:** The use of self-adhesive composites in dental treatment relatively a new concept. The aim of this study was to in vitro evaluation cytotoxicity of Vertise Flow and Nova Compo SF Flow self-adhesive flowable composites on bovine dental pulp-derived cells.

**Material and Method:** Experimental test samples (2×5mm) of Vertise Flow and Nova Compo SF Flow were prepared. Bovine dental pulp-derived cells were incubated in MEM-Alpha (Gibco-Invitrogen). The material samples to be tested were stored in the culture medium for 24 hours and therefore obtained extracts were applied onto the cells. The cell viability was determined by MTT assay. One-way ANOVA and Tukey-HSD post hoc tests used for statistical analysis.

**Results:** The percentage of cell viability of Vertise Flow found as 81.55%, Nova Compo SF Flow found as 71.40%. There is statistically significant difference between the control group and the test groups in term of the percentages of cell viability ( $p < 0.05$ ).

**Conclusion:** Self-adhesive flowable composites affect cell viability but they not have cytotoxic effects.

**Keywords:** Self-adhesive flowable composite, cytotoxicity, cell culture, MTT, bDPCs

## INTRODUCTION

Adhesive dentistry is developing rapidly every day. The first factor accelerating this development is the increasing demand of patients to aesthetic restorative materials. The other factor is that the physicians want to perform restorative procedures with minimum intervention in less time (1,2). The most commonly used restorative system in today's dentistry practice is the combination of adhesive resin/composite resin (1). Adhesive systems are mainly divided into two groups; 'etch & rinse' and 'self-etch' adhesives. Before placing the resin composite, etch & rinse adhesive system require acid etching, rinsing and drying as a first step then requires applying a priming agent and adhesive. This greatly prolongs the clinical application time (3). However, physicians must finish their restorations as soon as possible. Self-etch adhesives eliminate the acid etching and rinsing steps and contain weakly acidic monomers in the primer. It is therefore very popular now. In particular, single-step self-etch adhesive system that combines the etching, priming and adhesion in

one stage. Therefore, this system is very successful in shortening the clinical application time. However, even single-step self-etch adhesives have a considerable clinical application time and some technical sensitivity (4).

There are exciting advances in the development of restorative materials that can be directly adhered to the dental hard tissues without requiring any adhesive system. The first material produced for this purpose was Vertise Flow, Kerr which is a flowable composite. The self-adhesive flowable composite is a restorative material that is formed by adding a single-stage self-etch adhesive resin and applied directly to the cavity. It is based on the use of acidic monomers. HEMA monomer is another functional monomer which can be used in self-adhesive flowable composites (5). It is aimed to simplify restorative processes by eliminating the additional adhesive resin application phase by the using of self-adhesive flowable composites (6).

There are many materials used in the restorative treatment of decayed teeth. However, these materials help to restore the health of the tooth, they also may have the potential to produce undesirable effects on body tissues (7). In our days, advances in biomaterials focus on simplifying techniques improving material performance and improving biocompatibility and to achieve better results in less time (8,9). Since this material will be in close relationship with the pulp-dentin complex for a long time, its effects on pulp tissue is very important and should be investigated.

The aim of this in-vitro study was to evaluate the cytotoxicity of Vertise Flow and Nova Compo SF Flow self-adhesive flowable composites on bovine dental pulp-derived cells. Our null hypothesis is that self-adhesive flowable composites have no effect on cell viability.

## MATERIAL AND METHOD

The study was carried out with the permission of Selçuk University Faculty of Dentistry Ethics Committee (permission granted: 2020/60, decision no: 07). All procedures were performed adhered to the ethical rules and the Helsinki Declaration of principles.

Standardized cylindrical test samples with 2 mm height and 5 mm diameter of Vertise Flow, Kerr and Nova Compo SF Flow, Imicryl were prepared according to their manufacturers' instructions (n=15). The contents, lot numbers and manufacturers of the materials used in the study are shown in **Table**. The test samples stored in the culture medium for 24 hours and subsequently extracts obtained.

**Table.** The contents, Lot numbers and manufacturers of the self-adhesive flowable composites used in the study

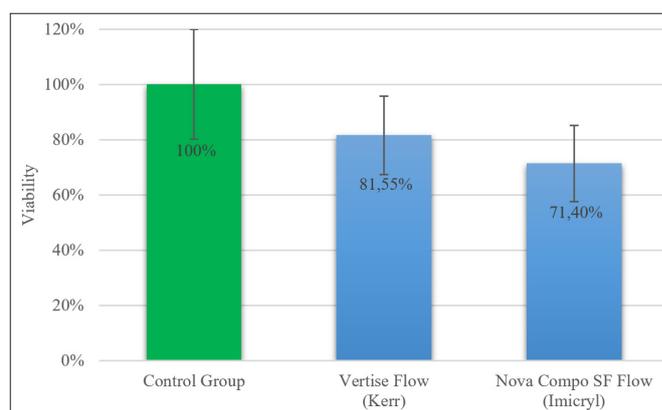
Materials	Content	Lot Number	Manufacturer and Country
Vertise Flow	GPDM, HEMA, 4-Methoxy phenol, Nano-ytterbium fluoride, barium glass, nano-size colloidal silica, zinc oxide, activator, stabilizer and colorants	3488779	Kerr, Germany
Nova Compo SF Flow	10-MDP, Bis-GPDM P, Bis-HEMA P, HEMA P, 4-META, 10-MDP + 4-META	2027A	Imicryl Turkey

bDPCs (bovine dental pulp-derived cells) were cultured in a 96-well plate which have MEM Alpha containing 20% FBS (fetal bovine serum), 1% geneticin and 5% penicillin/streptomycin at 37°C with humid air containing 5% CO<sub>2</sub>. Obtained test extracts applied to experiment group cells and original culture medium was used for control group. bDPCs viability was analyzed by measuring the

mitochondrial activity with the methyl tetrazolium assay (MTT) after 24 hours of exposure. The absorbance spectrophotometrically measured at 540 nm. The mean values of experiment groups proportioned to mean of control group. Therefore, viability value obtained as a percentage. One-way ANOVA and Tukey-HSD post hoc tests used for statistical analysis.

## RESULTS

The results of the cytotoxicity test are summarized in **Figure**. The percentage of cell viability of self-adhesive flowable composites were found as 81.55% (Vertise Flow), 71.40% (Nova Compo SF Flow) and 100% (Control) respectively. There is statistically significant difference between the control group and the test groups in term of the percentages of cell viability ( $p < 0.05$ ). Both Vertise Flow and Nova Compo SF Flow showed lower percentages of cell viability than the control group and there was no statistically significant difference among the percentages of cell viability of them ( $p > 0.05$ ).



**Figure.** Percentages of cell viability of bovine dental pulp-derived cells exposed to self-adhesive flowable composites.

## DISCUSSION

In minimally invasive dentistry composite resins, which are applied together with adhesive systems, stand out as the most preferred material. Due to the widespread use of composites over the years, the focus has been on shortening the application procedures of these materials and in this direction, self-bonding fluid composites have been introduced to the market (10).

All biomaterials used in dentistry must be evaluated for biocompatibility using screening assays to protect patient health and safety. Biocompatibility implies that a material does not cause systemic and local toxic, allergic, mutagenic and carcinogenic effects when in contact with vital tissues (9). Non-biocompatible or cytotoxic restorative materials can cause reactions ranging from short or long term post-

operative hypersensitivity to irreversible pulp damage (11). Many information on the harmful effects of the components of resin-based materials has been obtained from in vitro studies (12,13). Each material should be tested for biocompatibility before application on patients. Materials approved by independent researchers with biocompatibility are more reliable. In the investigation of biocompatibility, it should be preferred to use a standard, simple and quick test method. Cell culture assays, which are reliable, reproducible are frequently used to investigate biocompatibility (14,15). In our study, direct contact test method in which material extracts come into direct contact with cells was used and supportive results were obtained. Biocompatibility of dental materials can be measured with three types of biologic tests: in vitro, animal and usage tests (14). The in vitro MTT test has been shown to be a suitable in vitro method for assessing the cytotoxicity of dental materials. It has therefore become a standard test commonly used to assess the cytotoxicity of new biomaterials (16). Recently bovine dental pulp-derived cell line was developed for better mimicking of primary pulp cells by transfection with large T-antigen of SV40 (Simian Virus 40) (17). For these reasons, MTT assay and bovine dental pulp-derived cells were chosen as methods in our study.

One of the important ingredients self-adhesive flowable composite is glycerophosphate dimethacrylate (GPDM), which is a phosphate-based self-etch acidic functional monomer, and its task is to etch a rough surface required for preservation of enamel and dentin and to increase the wettability of this rough surface. Vajrabhaya et al. (18) evaluated the cytotoxicity of the Optibond Solo Plus SE (Kerr, USA), a dental adhesive containing GPDM as a functional monomer by dentine barrier test, and reported that the Optibond Solo Plus SE did not show cytotoxic effect. The other important functional monomer is hydroxy-ethyl methacrylate (HEMA). HEMA is a monomer which is added to many dental adhesive contents to increase wettability and to improve penetration of the resin into dentin. It has been shown that HEMA can be released from methacrylate-based resin composites and even physiological concentrations can affect pulp cells (19,20). On the other hand, HEMA, a small hydrophilic monomer, has been reported to diffuse even from sclerotic dentin (21). Pawlowska et al. (22) reported that HEMA could induce harmful biological effects such as DNA damage, apoptosis and delay in cell cycle. According to the results of a study conducted by Ülker et al. (23) residual monomers or other harmful components in Vertise Flow are difficult to diffuse from dentin and affect pulp cells. In this study, our null hypothesis is rejected. Although Vertise Flow and Nova Compo SF Flow decreased cell viability compared to the control group, it cannot be said that they have cytotoxic

effects since the cell viability is over 70% according to ISO 10993-5 standard (24). Also, the cell viability of Nova Compo SF Flow was less, no statistically significant difference was found between Vertise Flow and this group.

The clinical relevance of the in vitro data presented has to be interpreted with caution. Oral cavity condition differs from in vitro status and contains many factors such as saliva, mucus, creatine levels, food and drink intake, and normal flora. Only one method was used in this study to evaluate the cytotoxicity of self-adhesive composites, further studies may help to better understanding.

## CONCLUSION

In this study, it was determined that self-adhesive flowable composites affect cell viability but they not have cytotoxic effects. These materials are in close relationship with the pulp-dentin complex for a long time, so their effects on pulp tissue are very important and should be investigated by further studies.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** The study was carried out with the permission of Selçuk University Faculty of Dentistry Ethics Committee (permission granted: 2020/60, decision no: 07).

**Informed Consent:** For this type of study, formal consent is not required.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

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**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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