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# A New Generation of Sterile and Radiopaque Impression Materials—an *in vitro* Cytotoxicity Study

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**ABSTRACT:** Impression materials are largely used to record the geometry of dental tissue. Hence, the assessment of their possible cytotoxicity is a necessary step in the evaluation of their biocompatibility. The present study is carried out to evaluate the cytotoxicity of a new elastomeric sterile and radiopaque impression material. Human gingival fibroblasts, cultured *in vitro* are exposed directly to Elite Implant in three different viscosities, heavy, medium, and light. At 3, 9, 24, 48, and 72 h, the cellular proliferation is evaluated. In parallel, human gingival fibroblasts are exposed indirectly by means of fluid extracts of Elite Implant. The cellular viability is evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, (MTT) assay (Sigma, St Louis, Mo). The gingival fibroblasts proliferation and viability are unaffected by the presence of Elite Implant. This new impression material may represent a safe medical device for clinical and surgical applications. In addition, this material is radiopaque and, thus, can be identified radiographically.

**KEY WORDS:** dental impression material, human gingival fibroblast, *in vitro* cytotoxicity tests, proliferation, viability.

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## INTRODUCTION

**A**ll dental impression materials should accurately replicate intraoral and extraoral tissues in terms of accuracy, dimensional stability, elasticity, tear strength, rigidity, reproduction of detail, and biocompatibility [1]. ISO 10993 is used to determine the safety of medical devices by defining various types of biocompatibility tests. Cellular toxicity testing, which is required for all types of medical devices, is covered in ISO 10993-5 [2].

Four types of elastomers are extensively used: polysulfides, condensation silicones, polyethers, and vinyl polysiloxanes [3]. They are generally supplied in two paste forms, base and catalyst, and may be dispensed through an auto-mixing cartridge. Although polyethers present many advantages for clinical use, several disadvantages have been reported including allergic and toxic reactions, contact dermatitis, and gingivitis [4–11]. Vinyl polysiloxanes are more commonly used [3]. Various studies have reported contradictory levels of toxicity of vinyl polysiloxanes, classified as toxic [7–10], less toxic [8], or non-toxic [9–11]. The different grades of toxicity, evaluated *in vitro*, depend on the type of culture (primary cell or permanent cell lines), on the type of *in vitro* test (direct or indirect), and on the manufacturing processes of the materials.

The toxicity of dental impression materials is especially important when, during an intervention, a fragment becomes entrapped and remains within the gingival sulcus [12,13] under the suture, during impression making for implants or a surgical prosthesis. Fragments of impression materials have been found in soft tissues [14–20], under the periosteum [15,21–23], in the spongy bone [24–26], and in the maxillary sinus [27–30]. This can also occur in implant dentistry, particularly during second stage implant surgery or during single stage surgery. The retention of these fragments can induce a severe inflammatory reaction [15–17,20,22,26], which can result in implant failure.

An *in vivo* study recently reported postoperative problems following root canal treatment [20]. One week after filling, the patient presented with a painful swelling associated with the treated tooth. Following clinical examination, a thin piece of residual polyether impression material left in the tissues was identified as the possible cause of the swelling. This material was transparent, and not detected by radiographic examination. A clinical report highlights the importance of the radiopacity of any impression material for assisting in location and retrieval of accidentally embedded materials [23].

The purpose of this study was to investigate the cytotoxicity of a new vinyl polysiloxane, which is both sterile and radiopaque.

## MATERIALS AND METHODS

### Impression Materials

The heavy, medium, and light forms of Elite Implant (Zhermack, Badia Polesine, Rovigo, Italy), which are both sterile and radiopaque, were studied.

### Cell Culture

Human gingival fibroblasts were chosen because they represent a good simulation of the *in vivo* condition particularly in cases of impression material retention [31] in the gum.

Human gingival fibroblasts were isolated from biopsies of a healthy subject, after providing informed consent, who was undergoing gingivectomy of the molar region. Immediately after removal, the biopsies were placed in collection medium consisting of Hank's balanced salt solution (HBSS) (Gibco, Grand Island, New York), containing 250 UI/mL penicillin and 0.25 mg/mL streptomycin, 0.05 mg/mL gentamycin, and 0.0025 mg/mL amphotericin B (all from Gibco). The fibroblasts were grown as previously described [9,31–33]. The cells were cultured in Dulbecco's modification of Eagle's medium (DMEM) (Gibco), supplemented with 10% fetal bovine serum (FCS) (Gibco), 50 UI/mL penicillin, 50 µg/mL streptomycin, 2 mM L-glutamine (Gibco), and 1 mM Na piruvate (Sigma, St Louis, Mo) at 37°C in humidified atmosphere, 95% air, and 5% CO<sub>2</sub>. The culture reached confluence in 7 d and was then subcultured until the experiments were initiated. In the study, the cultures were used to the sixth passage.

### Measurements of Cytotoxicity

The direct and indirect tests of cytotoxicity were carried out following pre-defined methodology [8,33,34] with some modifications [9].

### Direct Cytotoxicity Test

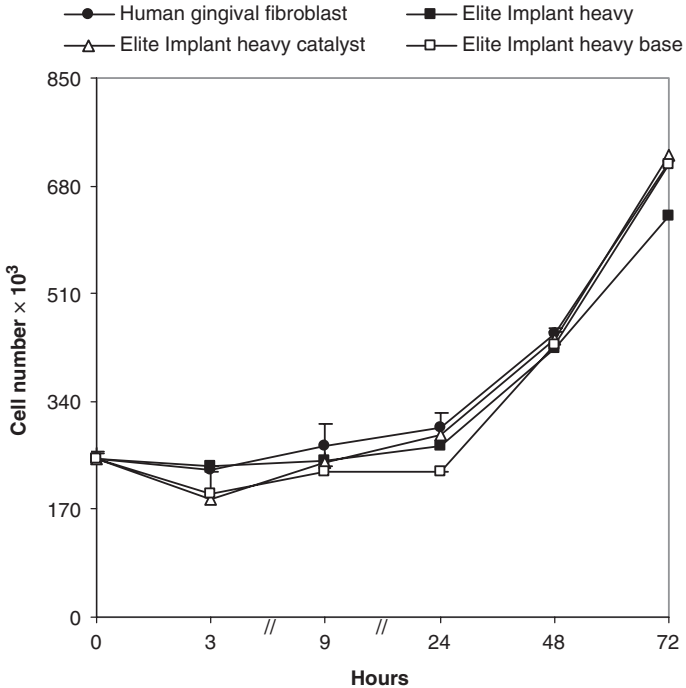
Human gingival fibroblasts,  $20 \times 10^4$ , were plated, in duplicate, in 60 mm petri dishes (Falcon, BD Bioscience, Milano, Italy) in a total volume of 5 mL of culture medium with 10% FCS and antibiotics, both in the presence and the absence of impression materials and their components. The polymerized impression materials (1 cm<sup>2</sup>) were

placed in the center of the petri dishes under sterile conditions. The dishes were incubated at 37°C under a humidified atmosphere of 95% air and 5% CO<sub>2</sub>. Cell proliferation was evaluated after 3, 9, 24, 48, and 72 h. At each time period the medium was removed, cells in monolayer were trypsinized (Na-EDTA trypsin) (Gibco) for 7–10 min, and counted in a hemocytometer (Neubauer). Data were analyzed statistically using Student's test and statistical significance was set up,  $p < 0.05$ . Reported values are the mean of four replicates  $\pm$  standard deviation.

### Indirect Cytotoxicity Test

The indirect test was performed by incubating impression materials (1 cm<sup>2</sup>) in petri dishes, in 5 mL of culture medium without FCS for 24 h at 37°C under sterile conditions. At the end of the incubation period, the soluble extracts of these impression materials were collected in tubes and enriched with 10% FCS (Gibco). Human gingival fibroblasts were plated at  $20 \times 10^3$  per well in 24-well plates (Falcon; BD Bioscience) in 2 mL of culture medium. When the cell cultures were sub-confluent, the medium was removed; the cell monolayer was washed with PBS and exposed to diluted (50%) or undiluted (100%) soluble extracts at 12 and 24 h. Each concentration was tested in quadruplicate wells together with appropriate controls. At the end of the treatment, cell viability was estimated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma, St Louis, Mo) [9,34]. The MTT assay is an indirect marker for cytotoxicity. It is based on the ability of cells to reduce the slightly yellow soluble MTT to a deep purple formazan product, insoluble in aqueous solution. The reaction is catalyzed by mitochondrial succinyl dehydrogenase.

After 24 h of cell culture in the presence of undiluted or diluted extracts of impression materials, the medium was removed and 2 mL of growth medium with 200  $\mu$ L of MTT (5 mg/mL in PBS) were added to the cultures. Cells were incubated, in the dark, at 37°C in a humidified atmosphere (95% air and 5% CO<sub>2</sub>) for 3 h. The growth medium was removed, 2 mL of dimethyl sulfoxide (DMSO; Sigma) were added to each well to dissolve the purple formazan. The absorbance was measured in a spectrophotometer at a wavelength of 540 nm. This parameter is an indicator of the functional integrity of the mitochondria, and hence of cellular viability. Reported values are the mean of four replicates  $\pm$  standard deviation and are expressed as percentages of the control values.



**Figure 1.** Direct effect of Elite Implant heavy viscosity polymerized and its components, base and catalyst pastes, on *in vitro* proliferation of human gingival fibroblasts. Each point represents the mean of four measurements  $\pm$  SD.

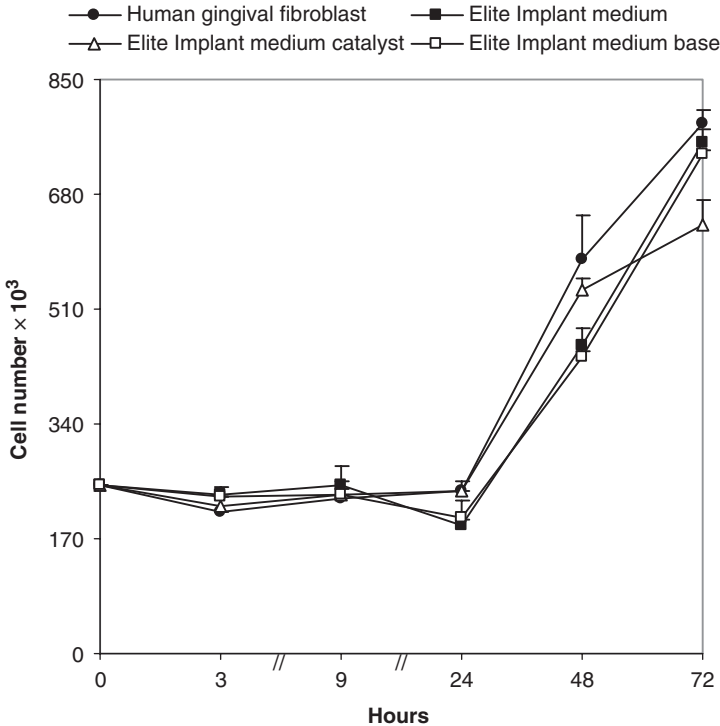
### Morphological Analysis

Human gingival fibroblasts were observed through an inverted microscope in phase contrast mode at  $\times 50$  magnification (Nikon Diaphot-TMD) and photographed after 3, 9, 24, 48, and 72 h of culture both in the presence and the absence of impression materials and their components.

## RESULTS

### Direct Effect of Elite Implant Polymerized and its Components – Base and Catalyst Pastes – On the Proliferation of Human Gingival Fibroblasts

The direct effect of polymerized Elite Implant heavy viscosity was evaluated at 3, 9, 24, 48, and 72 h in culture. The impression materials

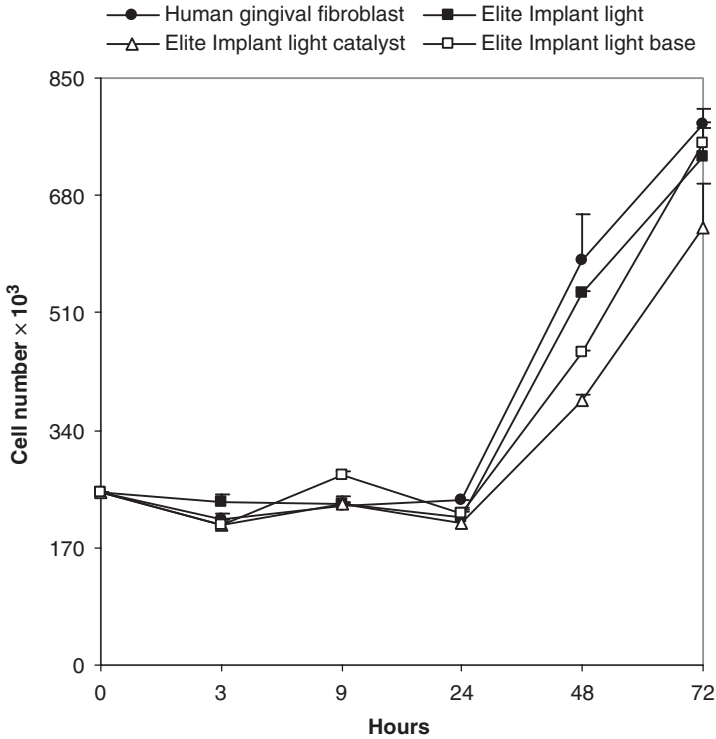


**Figure 2.** Direct effect of Elite Implant medium viscosity polymerized and its components, base and catalyst pastes, on *in vitro* proliferation of human gingival fibroblasts. Each point represents the mean of four measurements  $\pm$  SD.

did not inhibit cell proliferation at 48 h. At 72 h, Elite Implant heavy viscosity induced a negligible inhibitory effect,  $8 \pm 1\%$  lower than that found in control specimens (Figure 1). Elite Implant medium viscosity also produced a negligible inhibitory effect,  $13 \pm 4\%$  lower than that found in the control cells at 48 h, and at 72 h, the Elite Implant medium catalyst produced a clear inhibitory effect,  $\approx 19 \pm 5\%$  (Figure 2). Similar effects on cell proliferation were obtained by Elite Implant light viscosity and its components (Figure 3).

### **Indirect Effect of Elite Implant on the Viability of Human Gingival Fibroblasts Cultured *in vitro***

The diluted (50%) and undiluted (100%) extracts obtained after incubation for 24 h of the heavy, medium, and light Elite Implant materials did not induce any alteration in cellular viability (Figure 4).

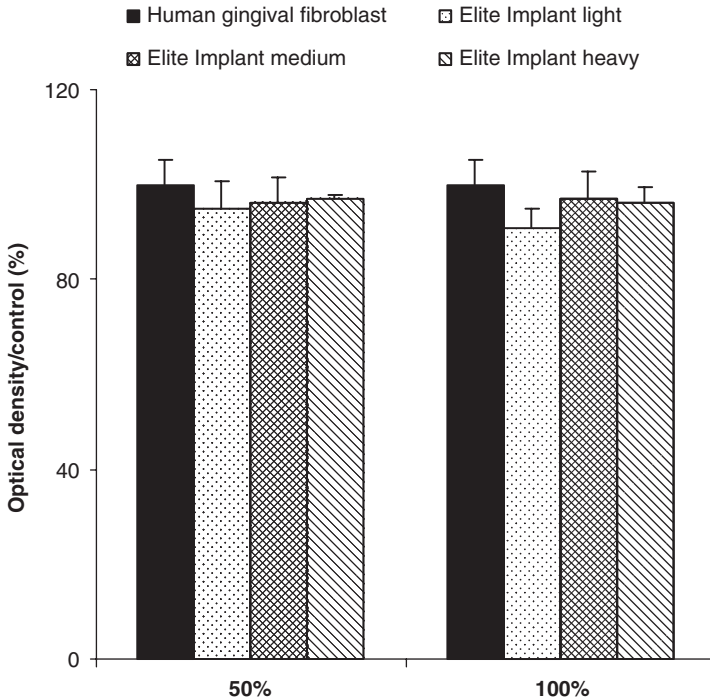


**Figure 3.** Direct effect of Elite Implant light viscosity polymerized and its components, base and catalyst pastes, on *in vitro* proliferation of human gingival fibroblasts. Each point represents the mean of four measurements  $\pm$  SD.

**Morphologic Analysis of Human Gingival Fibroblasts Cultured in the Presence of Elite Implant Heavy Viscosity Polymerized and its Components – Base and Catalyst Pastes**

At 48 h, human gingival fibroblasts, grown without impression material, were uniformly distributed and elongated in the petri dishes, showing typical fibroblast morphology (Figure 5A). In the presence of polymerized Elite Implant heavy viscosity (Figure 5B) and its components, base and catalyst pastes (Figure 5C and D), the culture of fibroblasts, did not show any morphological alteration. The growth and morphology of cells were similar to that of the control culture with some mitosis observed. At 72 h, the gingival fibroblasts were homogeneously distributed on the substrate and produced a complete monolayer. The cells increased in number and appeared with a narrow





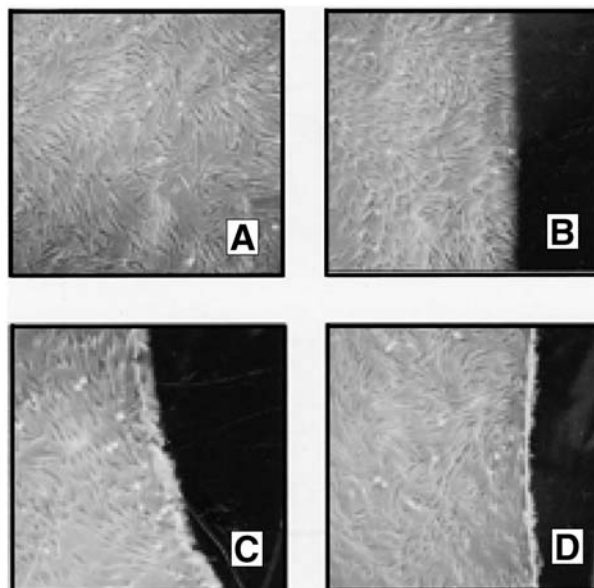
**Figure 4.** Indirect effect of diluted (50%) and undiluted (100%) extracts of Elite Implant heavy, medium, and light viscosity obtained after 24 h of incubation on cell viability of human gingival fibroblasts. Data are expressed as a percentage of optical density ( $\pm$ SD) compared with control cells (% of control).

and elongated morphology (Figure 6A). The morphology of gingival fibroblasts grown in the presence of Elite Implant Heavy viscosity polymerized and its components is similar to that of the control cells (Figure 6B–D).

## DISCUSSION

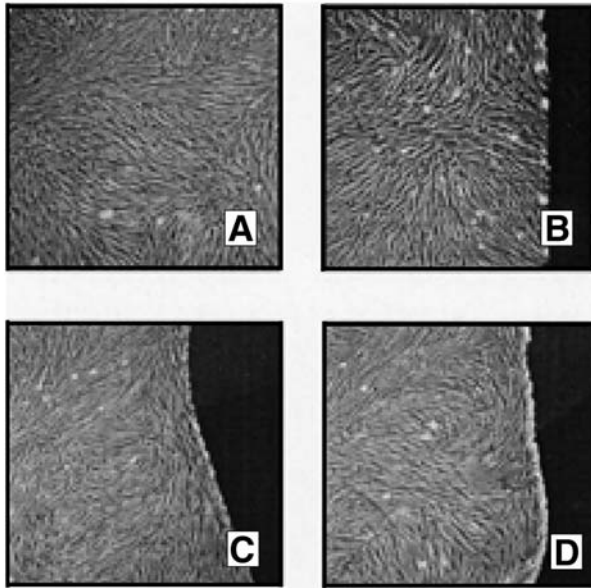
The aim of the present study was to evaluate the cytotoxicity of Elite Implant (Zhermack), a new elastomeric, sterile, and radiopaque impression material.

*In vitro* cytotoxicity tests were developed to simulate and predict biological reactions to the materials placed into or on human tissues. Considering this aim, particular care should be taken to select cell types and experimental conditions. Continuous cell lines are easy to



**Figure 5.** Morphological analysis of human gingival fibroblasts cultured for 48 h in the absence of impression material (A); in the presence of polymerized Elite Implant heavy viscosity (B); in the presence of base paste (C); and in the presence of catalyst paste (D). Cell cultures were observed by inverted microscopy in phase contrast mode at  $\times 50$  magnification.

maintain; they grow quickly and show good reproducibility. They are simple replicating systems without the specific metabolic potential that the target cells have *in vivo*. Moreover, continuous lines are sensitive and useful to test and classify the toxic effect of different materials [32]. In the present study, the cytotoxicity of this new dental material was assessed using human gingival fibroblasts cultured *in vitro*. These cells are characterized by a high degree of differentiation even if they are less homogeneous and sensitive than permanent cell lines; they are more comparable in their reaction pattern to oral mucosa. Human gingival fibroblasts retain specialized features and can represent a good simulation of the *in vivo* condition particularly in the case of impression material retention [31]. Elite Implant was found not to affect cellular proliferation and viability. This result confirms other reports [9,10,12] and also indicates that the manufacturing process and the components, added to the material in order to make it sterile and radiopaque, do not make it cytotoxic to



**Figure 6.** Morphological analysis of human gingival fibroblasts cultured for 72 h in the absence of impression material (A); in the presence of polymerized Elite Implant heavy viscosity (B); in the presence of base paste (C); and in the presence of catalyst paste (D). Cell cultures were observed by inverted microscopy in phase contrast mode at  $\times 50$  magnification.

human gingival fibroblasts. The cytotoxicity of Elite Implant was studied directly on gingival fibroblasts proliferation for 72 h. Generally, impression materials remain in contact with the oral tissues for a short time, typically a few minutes. Only in the situation of material retention in the oral tissues is the time of contact longer. In this case, a moderate or severe swelling of oral tissues may occur. If the impression material is radiopaque, the diagnosis of the swelling can be conducted with simple radiography [20–23].

According to the directive 93/42 CEE for medical devices [35], all non-invasive devices that come into contact with injured skin are classified into different classes. Class I includes the medical devices used as a mechanical barrier, for absorption of exudates. Class IIa includes medical devices intended to manage the micro-environment of a wound. Class IIb includes medical devices used principally in wounds which have breached the dermis and only heal by secondary intent. Elite Implant is the only elastomeric impression material classified in Class

Ila. Because of its sterility, it has an elective use in operating theaters, specific indications include regenerative bone surgery, intraoperative impressions, immediately loaded implant-supported prostheses, and when making impressions of sub-gingival implant platforms. Finally, the bright color (blue or yellow) of Elite Implant may help the dentist to visualize fragments left intraorally.

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