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Original Article

Biocompatibility and cytotoxicity assessment of 3D-printed orthodontic aligners: A comparative study of Graphy and LuxCreo resins on human periodontal ligament cells and osteosarcoma cells

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Abstract *Background/purpose:* The development of 3D-printed orthodontic aligners offers an alternative to conventional thermoformed aligners, reducing material waste and production time. However, concerns regarding biocompatibility and cytotoxicity remain. This study evaluates the cellular response of human periodontal ligament (PDL) cells and MG63 osteoblast-like cells to Graphy and LuxCreo 3D-printed resins, assessing their potential impact on orthodontic treatment and bone remodeling.

Materials and methods: PDL and MG63 cells were cultured and exposed to extracts from Graphy and LuxCreo resins. MTT assays measured cell viability at 24, 48, and 72 h, while phase-contrast microscopy analyzed cell morphology, adhesion, and confluency. Statistical comparisons were performed using ANOVA with Bonferroni post-hoc testing.

Results: Graphy-treated cells exhibited high viability and confluency, demonstrating progressive adaptation and proliferation. In contrast, LuxCreo-treated cells showed lower initial viability, with poor adhesion and slower recovery. MG63 cells, in particular, exhibited reduced

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osteoblast compatibility with LuxCreo, indicating potential limitations for bone remodeling applications.

Conclusion: Graphy demonstrated better biocompatibility and osteoblast support, making it a more suitable material for orthodontic applications. LuxCreo showed early cytotoxic effects, suggesting the need for surface modifications to enhance cellular response. Further research is required to optimize 3D-printed aligner materials for long-term intraoral use.

Introduction

The concept of using aligners to move and straighten teeth was first introduced by Harold D. Kesling in 1946.[1] In 1993, Sheridan et al. proposed the combination of interproximal reduction (IPR) with aligners to facilitate tooth alignment, particularly in cases where space was insufficient.[2] Align Technology (Align Technology, San Jose, CA, USA) was founded in 1997 by two Stanford MBA students and two orthodontists. In 1998, Align Technology developed the Invisalign system, the first CAD/CAM-based orthodontic appliance.

The use of orthodontic aligners has become increasingly popular across all age groups, demonstrating successful treatment outcomes. The mandibular advancement aligner (Align Technology) was specifically introduced for children with mandibular retrognathism malocclusion, functioning similarly to conventional functional appliances. Various materials are used in aligners, including polyethylene terephthalate-co-1,4-cyclohexylene dimethylene terephthalate (PETG), thermoplastic polyurethane (TPU), and copolyester polyethylene terephthalate (PET).[3]

Aligners are typically fabricated through a thermoforming process, which involves:

Intraoral scanning of the patient, digital alignment using software, model fabrication, thermoforming of the aligner, and delivery to the clinic. However, this thermoplastic fabrication process is time-consuming, labor-intensive, and costly, with high energy consumption, waste generation, and environmental impact.[4,5]

To address these limitations, additive manufacturing (3D printing) has emerged as an alternative fabrication method, allowing for the rapid production of aligners. The success of this method depends on the compatibility between printing parameters and materials, such as printer type, speed, and resin viscosity.[6,7] Digital light processing (DLP) 3D printing utilizes liquid photopolymer resins, and the degree of polymer conversion may influence cytotoxicity.[8,9] To ensure complete polymerization, external light-curing devices (LED/Xenon lamps) are often used.[10] Material cytotoxicity is also affected by the processing stage of the aligners.[11]

Additionally, intraoral aging of orthodontic appliance materials can impact their structural integrity, hydrolytic stability, and plasticization, leading to the release of chemical components into the oral environment. One of the most discussed substances in this context is bisphenol A (BPA).[12,13] Although manufacturers report compliance with safety standards, prior studies on Invisalign aligners have shown no significant cytotoxicity or chemical leaching.[14,15] Similarly, no major chemical composition changes have been observed between new and retrieved Invisalign aligners.[16] However, studies have reported elevated salivary BPA levels in patients using vacuum-formed polypropylene and polyethylene retainers, whether produced in-office or in laboratories.[17,18]

To minimize third-party dependency, costs, and delays, in-house 3D printing technology has been employed since 2021 as a cost-effective method for planning and fabricating orthodontic aligners.[19–22] While this approach shows promise, limited evidence exists regarding its biocompatibility. Early studies suggest that 3D-printed aligners possess acceptable mechanical properties, which remain stable after intraoral aging. However, concerns regarding their cytotoxicity persist. Given the long-term intraoral use of aligners, their safety must be carefully evaluated. Therefore, this study aims to assess the cytotoxicity of newly developed, in-office 3D-printable aligners.

Materials and methods

Cell culture

The PDLs and MG63 cells were obtained from Scien Cell Research Laboratories (Cat. #2630, Scien Cell Research Laboratories, Carlsbad, CA, USA). To culture HPDL cells, Dulbecco's Modified Eagle Medium (DMEM, Gibco, Thermo Fisher Inc., Waltham, MA, USA) was supplemented with 10 % fetal bovine serum (FBS, Thermo Fisher Inc.) and 1 % penicillin-streptomycin (100 U/mL). The cells were incubated at 37 °C in a 5 % CO_2 atmosphere for three days until they adhered to the culture dish, after which they were transferred for further passaging under the same conditions.

3D-printed resin aligner material preparation

Two 3D printing resins were used in this study: Tera Harz TC-85 DAC resin (Graphy, Seoul, South Korea) and LuxCreo resin (LuxCreo Inc., Chicago, IL, USA). LuxCreo specializes in digital dentistry, providing resins designed for chairside clinics and large-scale laboratory production. For details on the manufacturing process and comparison of the two brands, please refer to Table 1.

Orthodontic aligners were directly 3D-printed using Graphy and LuxCreo resins with a thickness of 0.75 mm.

Table 1 Comparison of 3D printing processes for clear aligners: Graphy vs. LuxCreo.

Comparison Item	Graphy	LuxCreo
3D printer used	Graphy Tera Harz/DLP 3D printers	LuxCreo iLux Pro/Lux 3+
Printing technology	DLP/SLA	LSPc
Printing material	TC-85DAP	Dental IBT/LT clear
Curing method	UV curing	UV + thermal curing
Curing time	20–30 min	UV 15–20 min + thermal 30–60 min
Additional processing	Oxygen barrier layer, polishing	Cleaning, heat treatment
Key features	Direct 3D printing of aligners, eliminating model casting	High transparency, enhanced mechanical strength

DLP: digital light processing, SLA: stereolithography apparatus, LSPc: light-sensitive printing continuous, UV: ultraviolet.

Following complete post-curing according to each manufacturer's protocol, the materials were shaped into 3.5 mm diameter discs using a punching technique on both the original 3D-printed sheets and thermoformed sheets.[23]

Cleaning and sterilization of 3D-printed samples for cell culture experiments

Before conducting cell culture experiments, 3D-printed samples undergo cleaning and sterilization to remove residual resin, suppress potential cytotoxicity, and ensure the accuracy of cell culture. The procedures are as follows:

Preliminary cleaning (removal of uncured resin):

Using absolute ethanol (≥95 %), place the sample into an ultrasonic cleaning machine and immerse it in the ethanol solution for 5–10 min, repeating the process 2–3 times. Remove the sample and transfer it into sterile PBS solution, gently shaking it for 5 min to eliminate any remaining organic solvents.

Sterilization to prevent cell culture contamination:

Ultraviolet (UV) sterilization is performed by placing the sample inside a sterile laminar flow hood and exposing it to UV light for 30–60 min to eliminate microbial contamination. The sterilized sample is then placed in cell culture medium for 72-h pre-treatment before proceeding with the experiment.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay for cell viability

The MTT assay (Sigma Aldrich Co., St. Louis, MO, USA) was conducted to evaluate cell viability. HPDL and MG63 cells were seeded into 24-well flat-bottomed tissue culture plates. After 24 h of incubation, the culture medium was replaced with 200 μL/well of the extract. After another 24-h incubation, the medium was replaced with 100 μL/well of MTT solution (1 mg/mL in PBS). The cells were incubated at 37 °C in a 5 % CO_2 atmosphere for 1 h. After incubation, the solution was removed, and 100 μL/well of dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. The plates were gently swirled for 10 min, and optical density (OD) was measured at 540 nm using a spectrophotometer (Sunrise Co., Männedorf, Zurich, Switzerland).

Cell viability (%) was calculated using the formula:

$$\text{Cell Viability} = \frac{\text{OD of test group}}{\text{OD of control group}} \text{X } 100$$

A survival rate above 70 % was considered safe for cell viability.

Microscopic analysis of cell morphology

After treatment with different materials, HPDL and MG63 cells were examined under a microscope at 100X, 200X, and 400X magnifications to assess cell morphology, adhesion, and proliferation.

Statistical analysis

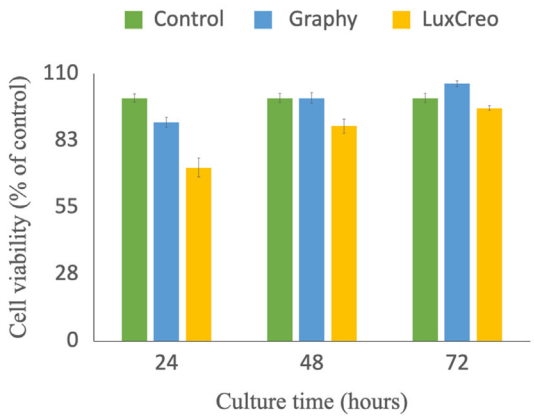
Statistical analysis was conducted using SPSS 22.0 (IBM, Armonk, NY, USA). Differences between mean values were analyzed using one-way analysis of variance (ANOVA) with Bonferroni post-hoc testing. A P-value <0.05 was considered statistically significant. To ensure data reliability, three independent experiments were performed in triplicate.

Results

Cell viability of PDL cells cultured on different materials

Fig. 1 illustrates the cell viability of PDL cells cultured on control, Graphy, and LuxCreo materials for 24, 48, and 72 h, expressed as a percentage relative to the control group. Graphy-treated cells showed an initial reduction in viability at 24 h (89.9 % ± 2.1 %), but fully recovered by 48 h (100 % ± 2.2 %). By 72 h, viability further increased to 105.9 % ± 1.3 %, surpassing the control group.

LuxCreo-treated cells displayed significantly lower viability at 24 h (71.3 % ± 4.0 %), suggesting initial cytotoxic effects. At 48 h, viability increased to 88.4 % ± 3.1 %, but remained lower than the control and Graphy groups. By 72 h, viability reached 95.8 % ± 1.1 %, approaching control levels. These results suggest that Graphy material promotes PDL cell adaptation and proliferation, whereas LuxCreo may require a longer adaptation period.



PDL cell	24 hours	48 hours	72 hours
Control	100 ± 1.8	100 ± 2.0	100 ± 2.1
Graphy	89.9 ± 2.1	100 ± 2.2	105.9 ± 1.3
LuxCreo	71.3 ± 4.0	88.4 ± 3.1	95.8 ± 1.1

Figure 1 Cell viability of PDL cells cultured on control, Graphy, and LuxCreo materials at 24, 48, and 72 h.

Morphological observations of PDL cells on different materials

Fig. 2 presents phase-contrast microscopic images of PDL cells on control, Graphy, and LuxCreo materials at 100X, 200X, and 400X magnifications. Control group: Elongated, spindle-shaped fibroblast morphology, forming a confluent monolayer by 72 h, indicating optimal biocompatibility. Graphy group: Initially, slightly lower cell density at 24 h, but normal morphology and confluency by 72 h, similar to the control. LuxCreo group: Reduced adhesion, rounder morphology, and lower cell density at 24 h. At 48 h, cell attachment improved but remained inferior to Graphy. By 72 h, cells adhered but did not form a fully confluent monolayer, suggesting suboptimal adhesion properties.

Cell viability of MG63 cells on different materials

Fig. 3 illustrates the viability of MG63 osteosarcoma cells on control, Graphy, and LuxCreo materials for 24, 48, and 72 h. Graphy-treated cells exhibited a moderate decrease in viability at 24 h (88.8 % ± 2.4 %), but recovered at 48 h (91.0 % ± 2.2 %), reaching 97.6 % ± 2.2 % at 72 h, indicating progressive adaptation. LuxCreo-treated cells showed a greater decline in viability, particularly at 48 h (82.6 % ± 1.7 %), with only minimal recovery at 72 h

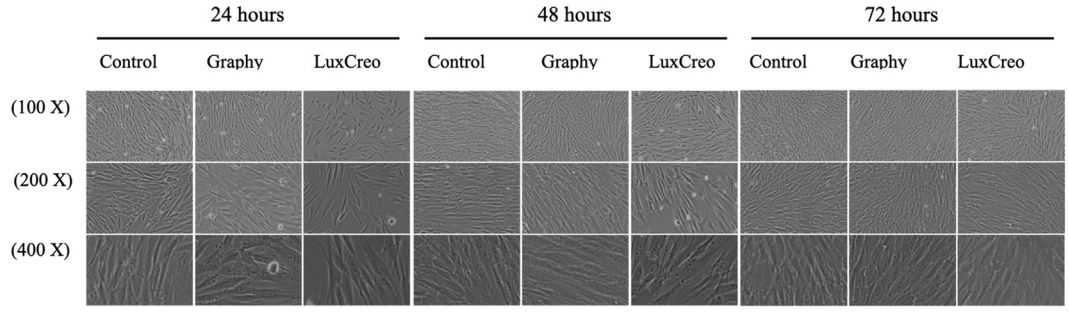
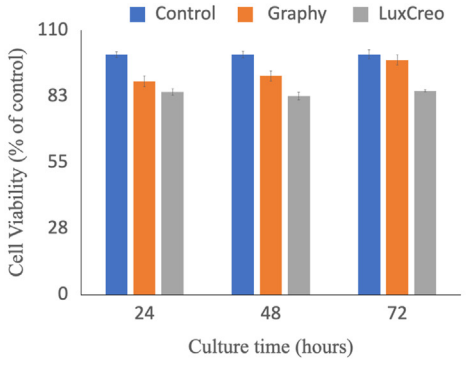


Figure 2 Phase contrast microscopic images of PDL cells cultured on control, Graphy, and LuxCreo materials at different time points and magnifications.



MG63 cells	24 hours	48 hours	72 hours
Control	100 ± 1.3	100 ± 1.5	100 ± 2.1
Graphy	88.8 ± 2.4	91.0 ± 2.2	97.6 ± 2.2
LuxCreo	84.3 ± 1.4	82.6 ± 1.7	84.8 ± 0.5

Figure 3 Cell viability of MG63 cells cultured on control, Graphy, and LuxCreo materials at 24, 48, and 72 h.

(84.8 % ± 0.5 %), suggesting weaker biocompatibility compared to Graphy.

Morphological observations of MG63 cells on different materials

Fig. 4 presents phase-contrast microscopic images of MG63 cells on control, Graphy, and LuxCreo materials at 100X, 200X, and 400X magnifications. Control group: Elongated, spindle-shaped osteoblast-like cells, forming a confluent monolayer by 72 h. Graphy group: Good adhesion and spreading, with minor density reduction at 24 h, but achieving full confluency by 72 h. LuxCreo group: Poor initial adhesion at 24 h, with rounder morphology and lower density. At 48 h, slight improvement was observed, but cell density remained low. By 72 h, cell numbers increased, but cells were scattered, with incomplete confluency, suggesting delayed adaptation.

Discussion

This study evaluates the biocompatibility of 3D-printed orthodontic aligner materials by analyzing their effects on PDL cells and MG63 osteoblast-like cells. Both cell types are crucial in orthodontic treatment, as PDL cells mediate mechanical force transduction, ensuring that orthodontic forces are transmitted to the alveolar bone, while MG63 cells regulate bone resorption and formation, influencing tooth movement and post-treatment bone stability.

The cell viability and morphological results (Figs. 1 and 2) indicate that Graphy material demonstrated high biocompatibility, with PDL cells adapting well and exhibiting enhanced proliferation over time. In contrast, LuxCreo-treated PDL cells showed lower initial viability and poor adhesion, suggesting early cytotoxicity. Although LuxCreo-supported cells recovered by 72 h, the delayed response implies suboptimal biocompatibility, which could negatively impact periodontal health during orthodontic treatment.

A biocompatible aligner material is essential for maintaining periodontal integrity, minimizing inflammatory responses, and ensuring efficient force transmission to the alveolar bone. The results suggest that Graphy is more suitable for long-term aligner use, while LuxCreo may require surface modifications to improve PDL cell attachment and proliferation.

The viability and morphological analysis of MG63 cells (Figs. 3 and 4) demonstrated a similar trend: Graphy-treated MG63 cells showed progressive adaptation, with viability and proliferation increasing over time, suggesting strong osteoblast compatibility. LuxCreo-treated MG63 cells exhibited consistently lower viability and incomplete confluency, indicating poor adhesion and delayed adaptation.

These findings imply that LuxCreo's surface characteristics or chemical composition may hinder osteoblast attachment, which could negatively impact bone remodeling during orthodontic treatment. Efficient orthodontic tooth movement requires balanced bone resorption and formation, and materials with poor osteoblast compatibility might delay bone adaptation and post-treatment stability.

The differences in cell adhesion, viability, and morphology indicate varying levels of biocompatibility between the two tested materials: Graphy material closely resembles the control, supporting better cell adhesion, spreading, and proliferation for both PDL and MG63 cells. LuxCreo material exhibited weaker cell compatibility, with initial cytotoxicity, poor adhesion, and lower viability, particularly for osteoblast-like MG63 cells. These findings align with previous research on thermoformed aligners, where some materials demonstrated mild-to-moderate cytotoxicity due to manufacturing processes and material composition.[24,25] In the context of 3D-printed aligners, post-curing procedures significantly influence biocompatibility.[26] A study reported that aligners post-cured using a nitrogen-based system were biocompatible, while those using alternative curing processes exhibited mild cytotoxicity.[27]

This study evaluated the cytotoxicity and biocompatibility of two 3D-printed orthodontic aligner materials (Graphy and LuxCreo) using PDL and MG63 cells. The results indicate that: Graphy material exhibits superior biocompatibility, with high PDL and MG63 cell viability, better adhesion, and enhanced proliferation. LuxCreo material demonstrated initial cytotoxicity, lower osteoblast viability, and delayed cell adaptation, suggesting suboptimal biocompatibility for orthodontic use. These findings provide valuable insights into the cytocompatibility of 3D-printed aligners. However, further research is required to optimize material properties and evaluate the long-term biological effects of 3D-printed aligners in clinical settings.

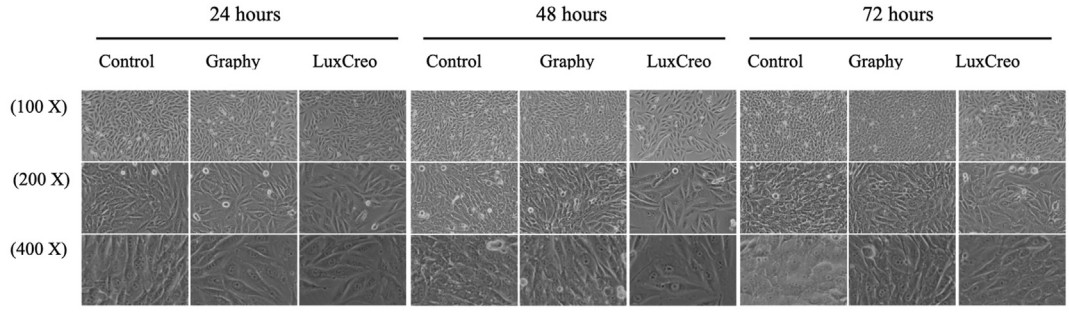


Figure 4 Phase contrast microscopic images of MG63 cells cultured on control, Graphy, and LuxCreo materials at different time points and magnifications.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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