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Effects of resin materials dedicated for additive manufacturing of temporary dental restorations on human gingival keratinocytes

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Abstract

Objective: This study investigated the effect of eluates of conventional and 3D-printed resin materials for manufacturing temporary dental restorations on gingival keratinocytes.

Methods: Three-dimensional (3D)-printed resin materials: 3Delta temp (Deltamed), NextDent MFH (Nextdent), Freeprint temp (Detax), GC temp (GC), were compared to Grandio disc (Voco) and Luxatemp (DMG). Human gingival keratinocytes (IHGKs) were exposed to eluates of the materials and XTT assays were performed at 24 h, 48 h, 72 h, or 144 h. For quantification of the proinflammatory response, the protein amount of IL-6 and 8 was determined in the supernatants using ELISA. One-way ANOVA with post hoc analysis was used to compare differences in cell viability and IL-6 and IL-8 levels between groups.

Results: At 24 h, and more remarkably at 48 h, a significant decrease in cell viability occurred for the 3D-printed materials compared to the untreated IHGKs, but also compared to Grandio disc and Luxatemp. Except for the expression of IL-8 in presence of the eluate of Grandio disc at 24 and 48 h, all tested materials caused attenuation of IL-6 and 8 from IHGKs for any observation period.

Conclusions: The materials for additive manufacturing affect cell proliferation differently than the subtractive manufactured material Grandio disc and the conventional material Luxatemp.

Clinical Significance: In comparison to conventional and subtractive manufactured restorations, 3D printed temporary restorations might induce more negative effects on the gingival and probably also on pulpal health since viability and the proinflammatory response of oral keratinocytes are more intensively affected by these materials.

KEYWORDS
3D-printing, additive manufacturing, biocompatibility, CAD-CAM, cytotoxicity

1 | INTRODUCTION

The technique of computer-aided design (CAD) and computer-aided manufacturing (CAM) has opened entirely new opportunities for the production of temporary and long-term dental restorations in the past decades. Most importantly, the subtractive manufacturing of dental workpieces enabled the routine employment of industrially fabricated high-quality materials providing standardized physical and chemical properties in dental restorative treatment. Recently, the potential of additive manufacturing (AM) to overcome several of the shortcomings of subtractive manufacturing has been reported, primarily focusing on the ability to fabricate even complex dental restorative objects within a single integrated production process, requiring, at the same time, smaller amounts of resin material.[1]

Additive manufacturing is commonly achieved by three-dimensional printing of a wide range of materials, i.e., resins, metal alloys, or ceramics.[2] Three-dimensional (3D) printing is essentially based on two different techniques, i.e., the stereolithography (SLA) and digital light processing (DLP) method. In both of these technologies, the printed object is constructed layer by layer within a light-curing material, for example, a photopolymerizing resin. Regarding the SLA method, the workpiece is created by curing the resin three-dimensionally with an ultraviolet laser beam. In terms of the DLP technique, the 3D shape of the printed workpiece is segmented into a series of single layers. For the polymerization of the material, a LED projector creates a single image of each layer across the building plate, leading to the polymerization of the entire layer at one time point. Yet, various resin-based materials suitable for 3D printing of crowns, bridges, on lays, and veneers have been developed.[3] Several recent in vitro studies have confirmed appropriate mechanical properties of these materials, specifically in terms of fracture strength and wear.[4,5] Different to subtractive manufacturing, using industrially prefabricated resin materials with highly standardized properties,[6,7] additive manufacturing creates resin-based workpieces with varying physical and chemical quality.[8]

Previous studies reported a very small amount of monomer release for materials used in subtractive manufacturing,[9] which is most likely explainable by the industrial fabrication and polymerization at high temperature and high pressure.[10]

For additive manufacturing, most recently, it has been shown that the release of monomers is considerably different, substantially depending on the particular resin material, handling, and 3D printing device.[11–14] This aspect assumes particular importance since dental restorations are commonly in intimate contact with oral hard and soft tissues. Apart from esthetical and functional aspects, provisional restorations are helpful in maintaining the shape of the gingival soft tissue and the emergence profile.[15]

It seems reasonable to assume that some of these monomers, as shown for conventional materials, influence the physiology of cells and cause adverse biological effects upon their release.[16–20] In fact, numerous studies have confirmed cytotoxic effects and gingival and oral mucosal reactions by some of these monomers.[21–25] In particular, negative effects on proliferation and viability have been evidenced.[26–29]

More recent studies have focused on alterations of IL-6 and IL-8 expression induced by monomers.[30–36] IL-8 is particularly expressed as part of the early response against inflammatory challenges of oral epithelial cells.[37] Additive manufacturing employs specific dental composites, developed exclusively for three-dimensional printing.[38] Resin materials suitable for 3D printing are primarily based on various organic methacrylate monomers, i.e., Urethane dimethacrylate (UDMA), tetraethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethyl methacrylate (HEMA).[39] For all of these monomers hazardous biological effects in terms of altering RNA synthesis and apoptosis in human gingival keratinocytes have been shown.[17,40–42]

At the moment, no information is available on the cell or tissue compatibility of 3D printed dental restorative materials, albeit these materials commonly reach close contact with the gingiva. Therefore, this in-vitro study aimed to investigate the biological effects of 3D printed resin materials used for the fabrication of dental temporary restorations on gingival keratinocytes. In particular, the study sought to assess the hypotheses that (1) 3D printed material does not affect cell proliferation nor would the manufacturing technique have any significant influence and (2) the expression of the proinflammatory cytokines interleukin 6 and 8 is not influenced by the different materials.

2 | MATERIAL AND METHODS

2.1 | Preparation of samples

Six resin composite materials were investigated in this trial, as summarized in Table 1. Four resin materials suitable for 3D printing, 3Delta temp (Deltamed, Friedberg, Germany), NextDent MFH (NextDent, Soesterberg, Netherlands), Freeprint temp (Detax, Ettlingen, Germany), GC temp (GC Europe, Leuven, Belgium) have been compared with one material used for subtractive manufacturing (Grandio disc; Voco, Cuxhaven, Germany) as a negative control, and one direct temporary material (Luxatemp; DMG, Hamburg, Germany) as a positive control. All specimens were manufactured according to the respective manufacturer's specifications.

A computer-assisted STL file was created to design standardized resin samples with a diameter of 20 mm and a thickness of 1.7 mm. Subsequently, the STL file for the printed materials was imported into the CAM software Netfabb Premium 2019 (Autodesk, Mill Valley, CA, USA), with samples positioned flat on the building platform. Slicing was performed according to the manufacturer's settings with a layer thickness of 50 μm for Freeprint temp, Nextdent MFH, GC temp, and 100 μm for 3Delta temp. All specimens were manufactured using a D20II DLP 3D printer (Rapidshape, Heimsheim, Germany) with the corresponding material printing parameters and post-processing specifications. Specimens made of Freeprint temp, Nextdent MFH, and GC temp were cleaned following the printing procedure for 2 minutes in an ultrasonic activated bath of 96% isopropanol, while 3Delta temp specimens were centrifuged for 3 min (Allegra X-15R, Beckmann-Coulter Life Science, Indianapolis, IN, USA) and rinsed with 96% isopropanol. Freeprint temp and 3Delta temp were post-cured for

TABLE 1 Brand names, manufacturers, composition (according to manufacturer) of materials tested in this study

Material	Manufacturer	Matrix	Inorganic fillers
3Delta temp	Deltamed, Friedberg, Germany	Methacrylates	Silicon dioxide; Dental glass (30% vol)
Nextdent c&b MFH	NextDent, Soesterberg, Netherlands	7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate; ethylene dimethacrylate; HEMA; TPO; E-BPA; Titanium dioxide; mequinol; 4-methoxyphenol; hydroquinone monomethyl ether	Silicon dioxide
Freeprint temp	Detax, Ettlingen, Germany	45- < 60 wt% Isopropylidenediphenol Peg-2 Dimethacrylat; 1- < 5 wt% 2 HEMA; 1- < 5 wt% TPO; 1- < 5 wt% HPMA; <1 wt% Phenyl-bis (2,4,6-trimethylbenzoyl)-phosphinoxid	–
GC temp Print	GC Europe, Leuven, Belgium	UDMA50- < 75%, TEGDMA10- < 25%; 4,4′-isopropylidenediphenol, ethoxylatedand 2-methylprop-2-enoic acid 2.5- < 5%; TPO 1- < 2.5%; 2-(2H-benzotriazol-2-yl)-p-cresol 0.1 < 0.2%	Quartz 10- < 25% vol
Grandio disc	Voco, Cuxhaven, Germany	14% UDMA+ DMA	Nanohybrid fillers 86% w/w
Luxatemp	DMG, Hamburg, Germany	UDMA; Aromatic dimethacrylate; Glycol methacrylate	Glass, Silica filler

2×2000 flashes under a nitrogen atmosphere (Otoflash G171, NK Optik, Baierbrunn, Germany). Nextdent MFH specimens were post-cured with LC-3D Print Box (Nextdent, Soesterberg, Netherlands) for 30 min. GC temp specimens were post-cured by Labolight DUO (GC Europe) for 6 min.

For samples manufactured with the subtractive method, the STL file was imported into the software InLab Cam 19.0, and cylinders with a diameter of 20 mm and a thickness of 16 mm were produced by a 5-axis CAD/CAM device (MCX5, Dentsply-Sirona, Bensheim, Germany). Cylinders were sliced afterward in 1.5 mm samples using a low-speed diamond saw (Isomet low-speed saw; Buehler, Germany) under constant water cooling.

The direct composite Luxatemp was inserted in a silicone mold, flattened, and then covered by a transparent glass plate, to obtain a smooth surface.

For every material, 3 biological replicates were used in each experiment, and 3 independent experiments were conducted. All samples were ground and polished under water cooling from their original thickness to 1.5 mm with diamond abrasive paper (until grit size P 1200) in a grinding system (EXAKT400CS, Exakt, Norderstedt, Germany) and visually inspected for a smooth surface without porosities and irregularities. To achieve appropriate disinfection, all composite samples were washed for 60s with ethanol 80% before testing.

2.2 | Cells

Immortalized human gingival keratinocytes (IHGKs) were obtained from a dental papilla from a patient undergoing oral surgery. The investigation has been approved by the Ethics committee of the Medical School of the University of Göttingen. IHGKs were isolated according to standard protocols. Initially, cells were cultured together with feeder cells (human gingival fibroblasts). After immortalization, transfecting hTERT according to published methods,[43] and SV40 according to standard protocols, the cells were cultured in a standard medium. These were maintained in Keratinocyte Growth Medium 2 (KGM2) (PromoCell, Heidelberg, Germany) supplemented with 1% Pen-Strep solution (Sigma–Aldrich, St. Louis, MO, USA) in a humidified atmosphere containing 5% CO_2, at 37°C. The medium was changed every 3 days. Cells grow till confluence.

2.3 | Preparation of eluates

The specimens prepared from each material, as described above, were immersed in KGM2 and incubated at 37°C for 24 h. The guidelines of ISO 10993-12 were used to determine the required amount of liquid per sample to be inserted. Accordingly, each specimen was immersed in 2.5 ml of medium per well in a 12-well. After 24 h the eluates were collected and the specimens were further immersed in 2.5 ml of KGM2 and incubated at 37°C for 3 days.

2.4 | Cell culture and cytotoxicity test

For this experiment, 5×10^4 IHGKs were seeded in 24-well plates, with a 0.5-ml cell culture medium in each well. They were pre-cultured in KGM2 for 6 days so that a confluence of 90% was achieved. The specimens were examined daily under inverted light microscopy (AXIO, Zeiss, Jena, Germany). On day 0, the KGM2 medium was substituted with 0.5 ml of the eluates obtained after 24 h from each material sample as described above. For the control, IHGKs were further cultured in KGM2. Four days were scheduled for the execution of each experiment: after 24 h, 48 h, 72 h, or 144 h. For each day, a

different 24-well plate was set. A medium change was performed on day 3, using the new eluates obtained from the further 3-day incubation of the specimens. Tetrazolium salt Cell Proliferation Kit II (XTT) (Roche Diagnostics, Mannheim, Germany) was added according to the manufacturer's protocols and the medium of each well was transferred to a 96-well plate. Varioskan (Thermo Fisher Scientific, Inc.) with the Varioskan software 3.00.7 were used for spectrophotometric analyses. Cell viability was registered as absolute activity and according to the ISO 10993-1/5 standards as relative activity normalized to the activity of cells of the control groups.[44]

2.5 | ELISA tests

ELISA (enzyme-linked immunosorbent assay) tests were performed to measure interleukin 6 (IL-6) and interleukin 8 (IL-8) levels in the cells of the supernatants. For this purpose, the Quantikine ELISA Human, respectively, for IL-6 and IL-8 (R&D Systems, Minneapolis, USA) was used. This is a heterogeneous quantitative sandwich enzyme immunoassay technique. The test involved the removal of cell supernatants after 0 h, 24 h, 48 h, 72 h, and 144 h of contact with the eluate of the different materials. The test was performed according to the manufacturer's instructions (R&D Systems Europe, 2015). Fifty microliters of each sample were used in each well of the ELISA plate and incubated for 2 h at room temperature. The optical density of each well was measured using an ELISA reader (Varioskan, Thermo Electron Corporation). The absorbance readings were made at 450 nm, with a wavelength correction at 540 nm.

2.6 | SEM analyses

The surface topography, roughness, and morphology of the cells were assessed by a high vacuum field emission scanning electron microscope (FE-SEM). Samples of each material were produced as above. Each specimen was inserted into a 24-well plate and 2.5×10^4 IHGKs were seeded on each, controlling the adhesion under an inverted light microscope (AXIO, Zeiss, Jena, Germany).

After 3 h of culture, the specimens were fixed overnight with 2% glutaraldehyde (at 4°C and dehydrated by incubation of 45 min each in a 5-step ethanol gradient (50%, 75%, 85%, 90%, and 100%; Carl Roth). The specimens were air-dried for 2 h at room temperature and finally sputtered with a gold/palladium alloy (3 × 60 s, Agar Sputter Coater) at a voltage of 5 kV. The surfaces were observed with different magnifications from x1000 to x3000. The images were digitally recorded using the field emission scanning electron microscope (Fe-SEM, ZEISS GEMINI, SUPRA 55VP, Carl Zeiss).

2.7 | Statistics

Statistical analyses were performed using SPSS (IBM, SPSS Statistics, version 26.0). The significance level was set at $\alpha = 0.05$. If not otherwise indicated all data are given as mean (±SD). Within each group, data were

tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Homogeneity of variance between groups was confirmed with the Levene test. Differences between groups were analyzed separately for each observation period using one-way ANOVA together with the post hoc Games-Howell test for multiple comparisons.

3 | RESULTS

3.1 | Cell viability

For all materials tested in this study, cytotoxic effects on IHGKs have been observed, obviously increasing for longer observation periods (Figure 1). The samples of the material for subtractive manufacturing (Grandio disc CAD/CAM) induced only minor differences compared to the control at 24 h ($p = 0.694$) and 48 h ($p = 0.997$). Considering cell viability at 24 and 48 h, samples of the conventional temporary material (Luxatemp) caused less impairment in comparison to all of the four 3D-printed materials at 24 and 48 h (p < 0.01). The viability was not statistically different from the Grandio disc at 24 h ($p = 0.544$) and 48 h ($p = 0.061$).

Considering cell viability according to the ISO 10993-1/5 standards samples of the materials Grandio disc, Luxatemp and Freeprint temp showed no cytotoxicity within the first 24 h (cell activity >75% reference), Nextdent MFH was slightly cytotoxic (50–75% reference), GC Temp moderate cytotoxic (25–50% reference) and 3Delta temp strong cytotoxic (<25% reference). At 48 h in samples of Grandio disc CAD/CAM and Luxatemp no cytotoxicity was found, Freeprint temp and Nextdent MFH showed moderate cytotoxicity, and 3Delta temp
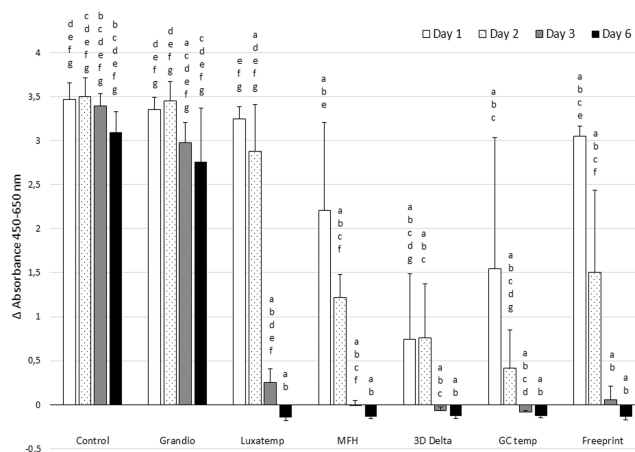

FIGURE 1 Tetrazolium salt reduction by IHGKs in the presence of eluates of different dental resin materials dedicated for temporary restorations: Grandio disc (Grandio), Luxatemp, Nextdent MFH, 3Delta temp, GC temp, Freeprint temp. Oral keratinocytes in a pure culture medium not exposed to eluates served as controls. Results are presented as mean values (± standard deviation). Differences between groups have been considered significant for p < 0.05 and marked above the respective column with the letters "a" material vs. control, "b" material vs Grandio disc, "c" vs Luxatemp, "d" vs. Nextdent MFH, "e" vs. 3Delta temp, "f" vs. GC temp, "g" vs. Freeprint temp
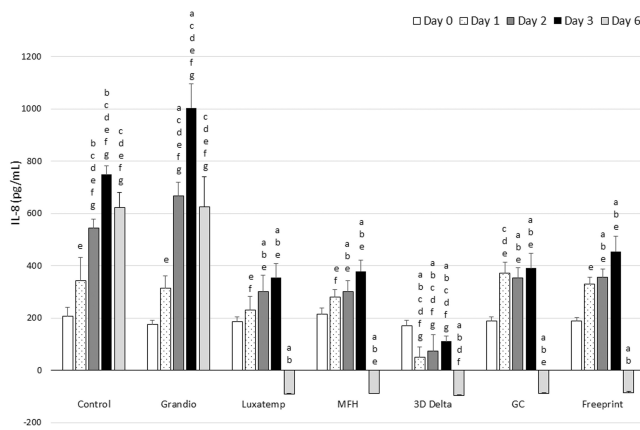
FIGURE 2 Expression of IL-8 by oral keratinocytes exposed to eluates of different dental resin materials dedicated for temporary restorations: Grandio disc (Grandio), Luxatemp, Nexdent MFH, 3Delta temp, GC temp, Freeprint temp. Oral keratinocytes in a pure culture medium not exposed to eluates served as controls. Results are presented as as mean values ± standard deviation. Differences between groups have been considered significant for $p < 0.05$ and marked above the respective column with the letters "a" material vs. control, "b" material vs Grandio disc, "c" vs Luxatemp, "d" vs. Nextdent MFH, "e" vs. 3Delta temp, "f" vs. GC temp, "g" vs. Freeprint temp.
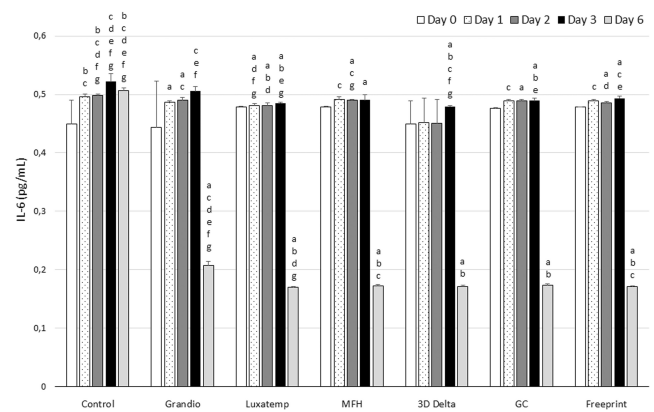

FIGURE 3 Expression of IL-6 by keratinocytes exposed to eluates of different dental resin materials dedicated for temporary restorations: Grandio disc (Grandio), Luxatemp, Nexdent MFH, 3Delta temp, GC temp, Freeprint temp. Oral keratinocytes in a pure culture medium not exposed to eluates served as controls. Results are presented as mean values ± standard deviation. Differences between groups have been considered significant for $p < 0.05$ and marked above the respective column with the letters "a" material vs. control, "b" material vs Grandio disc, "c" vs Luxatemp, "d" vs. Nextdent MFH, "e" vs. 3Delta temp, "f" vs. GC temp, "g" vs. Freeprint temp.

together with GC temp showed strong cytotoxic effects. Except for the negative control samples (Grandio disc), all materials tested herein induced strong cytotoxic effects at 72 h and 144 h (Figure 1).

3.2 | Expression of proinflammatory cytokines

The expression of interleukin 6 of IHGKs was attenuated following exposure against eluates of all of the tested materials compared to the untreated control samples for any of the observation periods (Figure 3). At 24 h, the difference was significant only for the subtractive manufactured material (Grandio disc; $p = 0.023$) and the conventional hand-mixed material (Luxatemp; $p = 0.002$). At 48, 72, and 144 h the difference in IL6 expression increased compared to untreated control samples, particularly for the 3D printed materials. Cells exposed to the eluate of the subtractive manufactured material showed almost similar expression of interleukin 8 at 24 and 144 h and a significantly stronger expression at 48 h ($p = 0.020$) and 72 h ($p = 0.009$) compared to the untreated control samples (Figure 2). Except for the material 3D Delta temp, all samples treated with eluates of the test materials and the untreated control samples showed increased expression of interleukin 8 for longer observation periods at 24, 48, and 72 h.

3.3 | SEM

SEM images of the polished surface texture of the different temporary materials show inorganic filler particles grouped together on the

surface of Luxatemp, 3Delta temp, and GC temp and less on Nextdent MFH. Moreover, a more porous surface was evidenced for 3Delta temp and GC temp. Filopodial extensions, indicative of a good cell spreading and adhesion on the surface of the material were evidenced just for the Grandio disc (Figure 4).

4 | DISCUSSION

Temporary restorations are of paramount importance in the clinical process of dental restorations. Various integral components of dental composite materials are released following the intraoral placement of the restoration, particularly monomers, that have been shown to cause significant biological effects on the cellular level, including cytotoxicity.[23,24] This appears to be even more important in cases of the use of temporary restorations particularly following surgical interventions, i.e., tooth removal or implant placement due to the close contact, at least partially, to tissue defects.

In terms of cytotoxicity of the resin materials designated for temporary dental restorations as tested herein, only the samples exposed to eluates of the subtractive manufactured material caused no impairment of cell viability during the entire observation period. This is probably due to the more efficient polymerization and low release of monomers.[9,10] Intriguingly, even the conventional temporary material that is commonly applied by hand mixing induced a considerably smaller decrease in cell viability in comparison to the four 3D printed resin materials.

Regarding the evaluation time, we decided to evaluate the acute response of the cells, thus evaluated daily until 72 h, and did not extend after 144 h, as already at this time point the vitality was
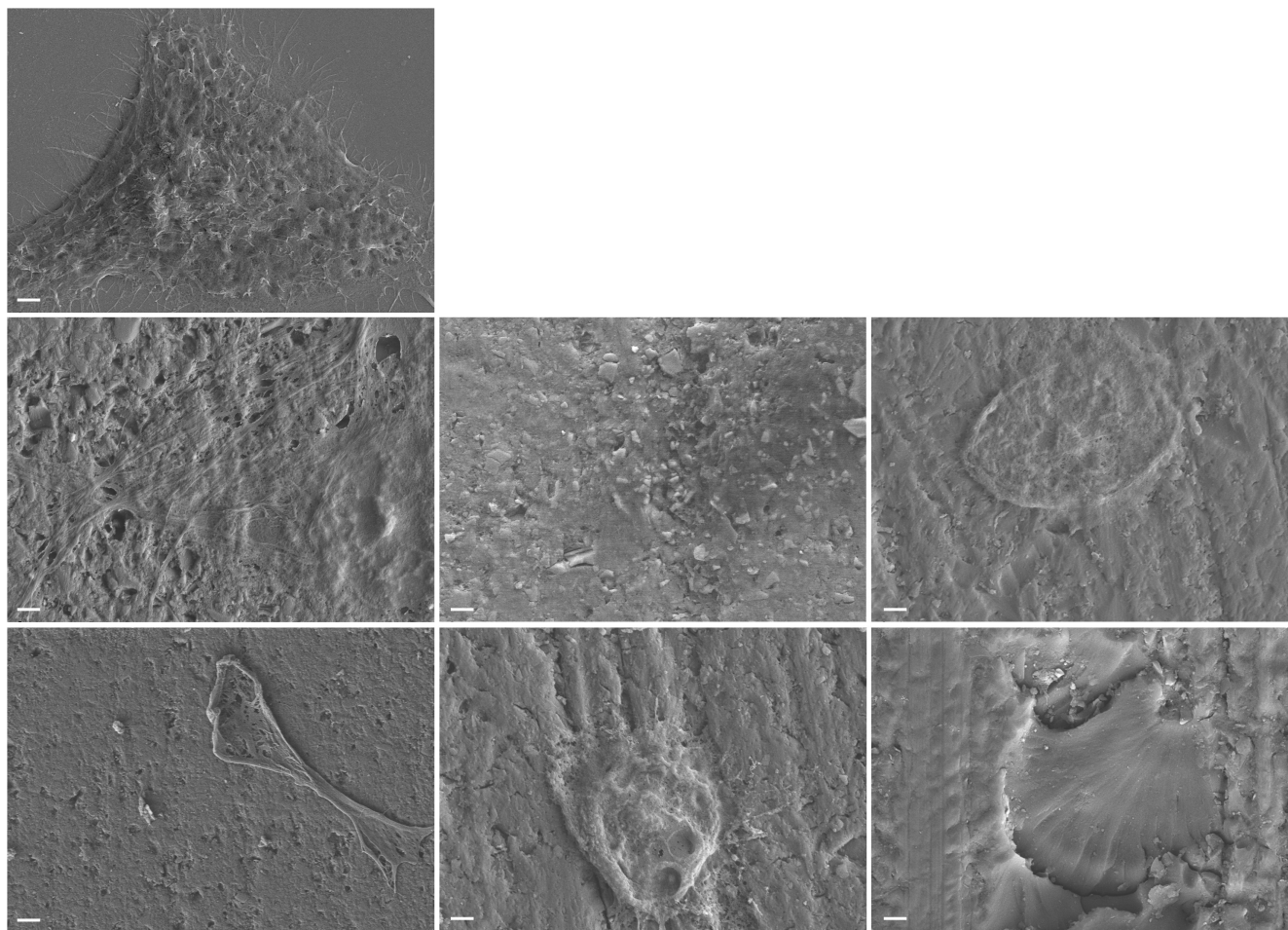
FIGURE 4 SEM images of surface textures of different material probes. Oral keratinocytes were cultured on the polished surface: a- keratinocytes on a glass plate, which served as control; b- Grandio disc; c- Luxatemp; d- Nextdent MFH; e- 3Delta temp; f- GC temp; g- Freeprint temp. Magnification x3000. The scale bars represent 2 μm.

compromised in most of the test groups. In this study, the XTT test was used to determine colorimetrically cell proliferation and viability. As a quantitative assay, the XTT test is commonly preferred over qualitative tests for the determination of cytotoxicity according to ISO standards.[45] It is based on the measurement of water-soluble formazan produced by dehydrogenase activity in the active mitochondria. The decrease in the number of living cells directly correlates to the amount of formazan formed, as monitored by the absorbance. Thus, cytotoxicity was indirectly evaluated.

Cytotoxicity of dental resin materials is primarily caused by leachable components, i.e., monomers and photoinitiators.[28,29] Hence, it appears likely that the lower monomer-to-polymer conversion rate in 3D printed materials than in conventional and industrial-manufactured dental resin materials is responsible for the current observations.[13] Different factors, including the chemical composition of the resin material, the relative amount of photoinitiators, and the handling during application together with the light exposure, determine the final degree of monomer conversion. Albeit the current study did not specifically address this issue, one might speculate that the additive manufacturing by 3D printing devices is much less

complete as compared to the conventional clinical and even more to the industrial manufacturing of dental resin materials.[14]

Focusing on the 3D-printed resin materials, cells exposed to eluates of Freeprint temp and Nextdent MFH showed only minor impairment of cell viability. According to the SEM analysis samples of Freeprint temp did not present with any filler particles within the surface. Due to improved transmission of the curing light, the absence of filler particles might enable a higher polymerization efficacy during the 3D printing process leading to a higher conversion rate and, ultimately, to a smaller amount of leachable monomers within the completed resin object. Due to the higher relative amount of filler particles of the 3D printed materials, 3Delta temp, and GC temp smaller sized monomers, i.e., TEGDMA and HEMA have to be included in order to achieve an appropriate viscosity of the material. In this regard, TEGDMA and HEMA show significantly stronger release into the water than other monomers commonly included in dental resin materials, specifically Bis-GMA and UDMA.[26] For both of these monomers, strong cytotoxicity has been proposed primarily mediated by the induction of reactive oxygen species.[25,27]

To protect cellular viability during the exposure of cells to resin monomers mainly three pathways are affected, i.e., the defense

against oxidative stress, inflammation, and the maintenance of the extracellular matrix.[35] Herein, the biological effects of the 3D printed resin materials and the expression of the proinflammatory cytokines IL-6 and IL-8 in presence of the eluates of the materials have been determined in addition to cell viability. Both cytokines have been widely used as marker molecules indicating inflammatory cell response associated with the exposure to dental composite materials.[18,19,36] Herein the expression of interleukin 6 and interleukin 8 was attenuated in presence of almost all resin materials with exception of the subtractive manufactured material, which caused stronger expression of interleukin 8 at 48 and 72 h. Partially in line, a previous study on conventional restorative composite resin showed a significantly reduced expression of interleukin 6 in mono-cultured oral keratinocytes after 24 h at the transcriptional and the protein level.[18,20] It also has to be noticed that the levels of inflammatory markers remain unchanged for 3D printed materials at 48 and 72 h, even though there is a decrease in viability of the cells. This means that the materials in these groups elicited a stronger expression of proinflammatory cytokines from the remaining cells. A recent in-vitro study on oral keratinocytes also found a significant reduction of the expression of both, IL-6 and IL-8 and its upstream regulator NFkB upon exposure to the hydrophilic resin monomer 2-Hydroxyethyl methacrylate (HEMA).[35] Further studies revealed partially inconsistent results, showing a material-dependent modulation of the inflammatory reaction by bulk-fill composites in pulp stem cells[22] and a clear induction of inflammatory mediators by conventional composites in human leukocytes.[34] Also various temporary resin-based dental materials had mixed effects on the expression of inflammatory mediators in human gingival fibroblasts.[33]

It has to be taken into account, however, that the cells in this study have been only indirectly exposed to the material using eluates obtained after 24 h of immersion of the resin specimens. We used this timeframe because according to several previous studies it was found that in the first 24 h most biologically active substances are released from the resin materials.[46,47] On the contrary, a study on dental composite using eluates after 24 h and 72 h of immersion of the material specimen found more pronounced effects, but the same trend of changes for eluates after 72 h of immersion.[18] Apart from the duration as used for immersion, the surface of all samples has been polished in the current study using a similar procedure as described in a previous study that showed comparable results as found herein.[22] Thus, it seems reasonable to assume that the differences observed between the 3D printed resin materials and the untreated control samples are in fact caused by the resin material itself rather than the polishing procedure.

A limitation of our study is related to the intrinsic limitations of a 2D cell culture model to address a clinical issue.

5 | CONCLUSIONS

Within the limitations of the current study, it was concluded that the resin materials tested herein, differently affected the viability of oral keratinocytes. Stronger effects were evidenced for the 3D printed materials in comparison to the conventional and the subtractive manufactured material. Based on the obtained results, the 3D printed resin materials seem to have a considerable impact also on the proinflammatory response of IHGKs.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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