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Cellular responses to 3D printed dental resins produced using a manufacturer recommended printer versus a third party printer

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PURPOSE. The aim of this study was to evaluate the influence of different 3D dental resins, using a manufacturer recommended printer and a third-party printer, on cellular responses of human gingival cells. **MATERIALS AND METHODS.** Three NextDent resins (Denture 3D+, C&B MFH and Crowntec) were used to produce specimens on printers NextDent 5100 (groups ND, NC and NT, respectively) and Phrozen Sonic Mini 4K (groups PD, PC and PT, respectively). Human gingival fibroblasts were cultured and biocompatibility was evaluated on days 1, 3 and 7. IL-6 and IL-8 concentrations were evaluated at 3 days using ELISA. Surface roughness was evaluated by a contact profilometer. SEM and fluorescence micrographs were analyzed at days 1 and 7. Statistical analyses were performed using SPSS and mean differences were tested using ANOVA and post-hoc Tukey tests ($P < .05$). **RESULTS.** There was an increase in cellular viability after 7 days in groups PC and PT, when compared to group PD. ND group resulted in higher concentration of IL-6 when compared to PT group. SEM and fluorescence micrographs showed less adhesion and thinner morphology of fibroblasts from group PD. No significant differences were found regarding surface roughness. **CONCLUSION.** The use of different printers or resins did not seem to influence surface roughness. NextDent 5100 and Phrozen Sonic Mini 4K produced resins with similar cellular responses in human gingival fibroblasts. However, Denture 3D+ resin resulted in significantly lower biocompatibility, when compared to C&B MFH and Crowntec resins. Further testing is required to support its long-term use, required for complete dentures. [J Adv Prosthodont 2024;16:126-38]

KEYWORDS
CAD-CAM; 3D printing; Biocompatibility; Resins; Surface roughness

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INTRODUCTION

Computer-aided design and computer-aided manufacturing (CAD-CAM) systems have a wide range of applications in dentistry, including orthodontics (for example, clear aligners), implantology (production of surgical guides) and production of indirect restorations and prostheses.[1,2] Nowadays, these techniques effectively reduce chair time and allow the storage of information through a digital file, for further usage.[3-5] There are two main different CAM approaches: subtractive manufacturing (SM) and additive manufacturing (AM).[6,7]

In SM, a pre-polymerized block of resin or other material is milled, but a significant amount of material is wasted. In AM, also known as 3D printing, objects are manufactured layer by layer, rendering it a more cost-effective procedure, requiring less expensive equipment and allowing the manufacturing of several pieces simultaneously.[6,8-11] Some devices are produced by AM technologies, such as occlusal splints, individual impression trays, models, and surgical guides, as well as, more recently, removable prothesis and temporary fixed prosthesis. Additionally, metallic Co-Cr frameworks of partial dentures and complete dentures (CDs) may be produced through laser sintering 3D printers.[12,13]

Most 3D printed materials used in AM are based on acrylic resins (monomethacrylates) or composite resins (dimethacrylates), with the composition being proprietary to the manufacturer.[14]

The intaglio surface is a key aspect for the adaptation of the CDs to the soft tissues. An adequate intaglio surface reduces trauma on soft tissue, minimizing bone resorption and improving comfort. For this reason, there are many studies that compare the intaglio surface of milled and 3D printed dentures, with similar results for both techniques.[15-17]

There is a lack of studies regarding behavior of different 3D printed materials and printers on the oral cavity, as mentioned in various articles.[9,10,18,19] A systematic review from Srinivasan *et al*.,[20] which evaluated various parameters of CAD-CAM dentures, including biocompatibility, only referenced 1 study reporting biocompatibility of 3D denture base material, which found no difference between the milled groups and 3D printed groups. A biocompatibility assay by Srinivasan *et al*.[21] was conducted in 2021, which concluded that milled and 3D printed resins had similar biocompatible results.

Related with the material and production technique, there are possible cytotoxic effects by the use of CDs, with the occurrence of contact stomatitis via irritant or allergic reactions, caused by residual monomers or specific components in the resin. Additionally, residual monomer can cause burning sensations in the mouth, oral ulcerations and oral lichenoid reactions. For this reason, biocompatibility must be assessed to ensure patient safety.[22-25]

This study was conducted based on the rationale that AM is considered a comparable alternative to SM and has several advantages, but according to many authors there is a lack of biocompatibility studies regarding the materials used and different methods of production. Additionally, only one study exists comparing the cellular responses to 3D printed dental resins using a manufacturer recommended printer and a third party printer, which used the Rapid ShapeD30 and the Form 2 printers, respectively. The third party printer, however, is a flagship 3D printer and in the particular case of this study, an expensive equipment in the context of end-user 3D printer scenario. Our study intended to evaluate the cellular responses to 3D printed dental resins using a manufacturer recommended printer and a less expensive third-party printer. The chosen cell culture is of importance, since dentures are in intimate contact with the mucosa, whose dominant cell type in the connective tissue is gingival fibroblasts.[26]

The aim of this study was to evaluate the influence of different 3D printers and resins on fibroblasts behavior. As the primary null hypothesis, we considered that the use of different 3D printers with equivalent parameterization does not influence the *in vitro* cellular behavior of human fibroblasts. Secondary null hypothesis considered that use of different resins does not influence the *in vitro* cellular behavior of human fibroblasts.

MATERIALS AND METHODS

Three resins were used in each group: Denture 3D+ in

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the shade Translucent Pink (lot number WY213N01, NextDent, Soesterberg, Netherlands), NextDent C&B MFH in the shade N1 (lot number EX433N03, NextDent, Zeist, Netherlands) and Crowntec in the shade A2 (lot number E276, SAREMCO, Rebstein, Switzerland). They were produced by two different printers: NextDent 5100 (3D Systems, Rock Hill, SC, USA) and Phrozen Sonic Mini 4K (Phrozen Technology, Hsinchu, Taiwan). 20 disc-shaped specimens were produced for each group with 8 mm of diameter and 3 mm of thickness. Specimen allocation is seen in Table 1 and chemical composition of the resins is seen in Table 2.

Specimen production was performed according to the resin manufacturer instructions for both printers, with a thickness of 50 µm in z axis in each layer and vertical orientation on the build platform. Both printers have rapid prototyping technology.

After specimen production, they were removed from the build platforms and the post-polymerization protocol was performed according to the manufacturer's indications for each resin. In short, specimens were washed with 96% ethanol for three minutes in an ultrasonic bath. The solution was then renewed, and the specimens were again submerged in 96% ethanol for an additional two minutes (the total time in the ethanol bath must not exceed five minutes). After the discs were dried for ten minutes, the final curing process was carried out using the NextDent LC-3D Print Box (NextDent®, Soesterberg, The Netherlands) for thirty minutes. LC-3D print box was turned on for 15 minutes previous to its use to ensure optimal working conditions.

Table 1. Allocation of specimens through the six groups (ND, NC, NT, PD, PC and PT), according to the designated printer and resin to be used during production

Group	3D Printer	Resin
ND[1]	NextDent 5100	Denture 3D+®
NC[2]		C&B MFH®
NT[3]		Crowntec®
PD[4]	Phrozen Sonic Mini 4K	Denture 3D+®
PC[5]		C&B MFH®
PT[6]		Crowntec®

Human gingival fibroblasts (HGF-hTERT) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Lonza, Visp, Switzerland), supplemented with 10% fetal bovine serum (FBS) (Biowest, Nuallié, France) and 1% of penicillin/streptomycin (Lonza, Visp, Switzerland).

For this study, five specimens of each group were decontaminated, placed in 48-well plate (Corning Inc®, Corning®, New York, NY, USA) and seeded at a density of 5×10^3 cells per well. A negative control consisting of cells at the same density seeded on empty wells was used in all assays. Three cell culture assays were executed to evaluate cellular biocompatibility (total n = 15).

Cellular viability and proliferation were evaluated with a Cell-TiterBlue® reagent (Promega®, Madison, WI, USA), by resazurin reduction test, according to the manufacturer's protocol. The conversion rate of non-fluorescent blue dye (only possible in viable cell mitochondria) was determined as fluorescence intensity in arbitrary fluorescence units (AU) after 1, 3 and 7 days of culture.

A multimode microplate reader (VICTOR Nivo™ HH3500, PerkinElmer®, Pontyclun, UK) was used to determine the fluorescence intensity, detecting excitation wavelengths of 530/30 nm and emission of 595/10 nm.

In order to quantify the interleukin 8 (IL-8) and interleukin 6 (IL-6) present in the cell culture supernatant, the Human IL-8 /CXCL8 DuoSet ELISA kit and Human IL-6 DuoSet ELISA kit (R&D Systems Inc®, Minneapolis, MN, USA) were used, according to the manufacturer's instructions, being measured at 72 hours of culture.

The optical density (absorbance) of the standard values and samples was measured using a multimode microplate reader (VICTOR Nivo™ HH3500) at 450 nm and 540 nm wavelengths, with the values obtained with the wavelength of 540 nm subtracted from those of 450 nm, in order to minimize interference optics in plate reading.

Based on the linear regression of the absorbance values recorded for the calibration curve, concentration of IL-8 and IL-6 in pg/mL were calculated.

The specimens were decontaminated, sterilized, seeded with HGF-hTERT (under the same conditions

Table 2. Material composition provided by resin manufacturer

Resin	Ingredient	% w/w	Classification according to Regulation (EC) No. 1272/2008 [CLP]
NextDent Denture 3D+®	Ethoxylated bisphenol A dimethacrylate	> 75	Aquatic Chronic 4, H413
	7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate	10 - 20	Skin Sens. 1B, H317 Aquatic Chronic 2, H411
	2-hydroxyethyl methacrylate	5 - 10	Eye Irrit. 2, H319 Skin Sens. 1, H317
	Silicon dioxide	5 - 10	Not classified
	diphenyl(2,4,6- trimethylbenzoyl)phosphine oxide (TPO)	1 - 5	Skin Sens. 1B, H317 Repr. 2, H361f Aquatic Chronic 2, H411
	Titanium dioxide	< 0.1	Not classified
NextDent C&B MFH®	7,7,9(or 7,9,9)-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bismethacrylate	50 - 75	Skin Sens. 1B, H317 Aquatic Chronic 2, H411
	2-hydroxyethyl methacrylate	< 25	Eye Irrit. 2, H319 Skin Sens. 1, H317
	Silicon dioxide	1 - 5	Not classified
	diphenyl(2,4,6- trimethylbenzoyl)phosphine oxide (TPO)	1 - 5	Skin Sens. 1B, H317 Repr. 2, H361f Aquatic Chronic 2, H411
	Ethoxylated bisphenol A dimethacrylate	< 10	Aquatic Chronic 4, H413
	Ethylene dimethacrylate	< 10	STOT SE 3, H335 Skin Sens. 1, H317
	Titanium dioxide	< 0.1	Not classified
	Mequinol 4-methoxyphenol Hydroquinone monomethyl ether	< 0.1	Acute Tox. 4 (Oral), H302 Eye Irrit. 2, H319 Skin Sens. 1, H317 Repr. 2, H361d Aquatic Chronic 3, H412
Crowntec®	BisEMA	50 - 75	Skin Irrit. 2, H315 Eye Irrit. 2, H319 Skin Sens. 1, H317 STOT SE 3, H335
	Trimethylbenzonyldiphenylphosphine oxide	0.1 - 1%	Repr. 2, H361 Aquatic Chronic 3, H412

previously mentioned), and fixated at 1 and 7 days of growth, for observation by fluorescence microscopy and scanning electron microscopy (FEG-SEM).

To evaluate possible changes in cellular morphology, fluorescence microscopy was used, in which the samples were initially washed with filtered PBS (VWR®, Philadelphia, PA, USA) and fixated with formaldehyde (PancreacApplichem, ITW Reagents Division, Darmstadt, Germany) at 4% for ten minutes. After the fixation process, the samples were washed again with filtered PBS. The cells were then permeabilized with 0.10% Triton X-100® (Merck KGaA, Darmstadt, Germany) for five minutes, after which the samples were washed with PBS. To stain the cytoplasm, a solution of Phalloidin (Phalloidin FITC Reagent - ab235137, Abcam, Waltham, MA, USA) was used and, to stain the nucleus, a solution of Propidium Iodide (Merck KGaA) was used.

For FEG-SEM, the samples were initially washed with PBS and fixated with glutaraldehyde (Electron Microscopy Sciences, Hatfield, UK) at 2.5% for one hour. After the fixation process, the samples were

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washed again with filtered PBS and the process of dehydration was carried out, using successively higher concentrations (from 20 to 100%) of ethanol (Honeywell Riedel-de Haën, Seelze, Germany), each incubated for thirty minutes. After the last concentration of ethanol, the solution was aspirated, and the samples were allowed to dry in the airflow chamber under UV light.

On the day of scanning electron microscopy observation, an ultra thin (15 nm) gold-palladium (Au-Pd) film of 80 - 20% mass was placed over the samples, through a high-resolution sputtering applicator (208HR Cressington Company, Watford, UK), coupled to a high-resolution thickness controller (MTM-20 Cressington).

Contact profilometry was used to measure the surface roughness of one specimen of each group and was performed by the Tencor® Alpha-step 200 Profilometer in the INESC MN facilities. The scanning stylus had 12.5 µm radius, a distance of 400 µm and a tracking force of 11 mg. Each specimen was measured in three to four points and the average roughness (R_a) was measured in KA and converted to µm.

Statistical analysis was performed using IBM® SPSS® Statistics 28.0 for macOS (SPSS, Chicago, IL, USA) and GraphPad Prism 9 for macOS (GraphPad Software Inc., San Diego, CA, USA).

Normality distribution was assessed for all samples using Kolgromov-Smirnov test.

Comparison among the groups for cellular viability, IL-6, IL-8 and surface roughness were performed based on one-way analysis of variance (ANOVA), using to post-hoc Tukey tests to identify significant differences among the groups. The significance level was defined as $P < .05$ and all results were presented as a mean ± standard deviation (SD).

RESULTS

According to the results shown in Fig. 1A, all groups presented an increased cellular viability over time.

At day 3, significant differences were found between the ND group and the PT group ($P = .013$). At day 7, significant differences were found between the PD group and the PC group ($P = .028$), and also between the PD group and the PT group ($P = .023$).

No differences were observed between different printer systems, specifically between the groups using NextDent 5100 printer and the groups using Phrozen Sonic Mini 4K printer, at any point in time (Fig. 1B).

However, significant differences were observed between different resins intended for 3D printing, as seen in Fig. 1C. Specifically, a significant decrease in viability was observed between the Denture 3D+ res-

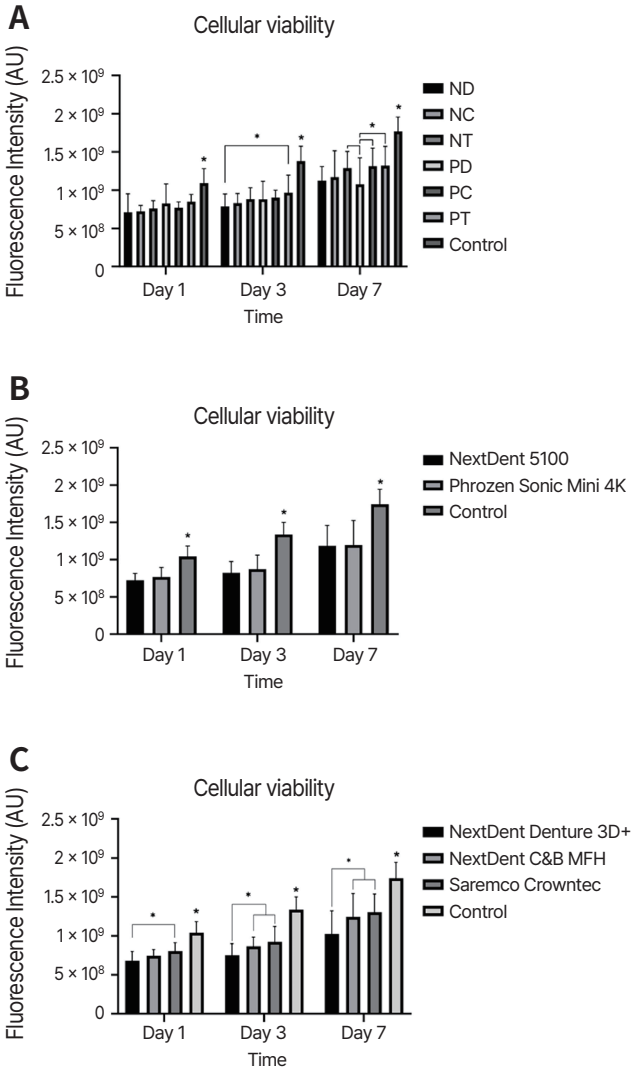

Fig. 1. Bar chart depicting cellular viability results as compound means ± SD in AU from Groups ND, NC, NT, PD, PC, PT and a negative control at 1, 3 and 7 days of culture (n = 15).

in and the Crowntec resin after 1 day ($P < .001$), while at day 3 and day 7, there was a significant decrease in viability of the Denture 3D+ resin group, comparing to C&B MFH ($P = .008$ at day 3 and $P = .002$ at day 7) and Crowntec resin groups ($P < .001$ at day 3 and 7).

Regarding the presence of IL-6 present in the cell culture supernatant, according to Fig. 2A, all groups had different concentrations of IL-6 at day 3. Significant differences were found between the ND group and the PT group ($P = .032$), with ND group significantly resulting in a higher concentration of IL-6.

No differences were observed between the two printers (as seen in Fig. 2B).

However, significant differences were observed among different resins, as seen in figure 2C, with Denture 3D+ resin resulting in a significantly higher concentration of IL-6, when compared to Crowntec resin ($P = .006$).

Regarding the presence of IL-8 present in the cell culture supernatant, according to Fig. 3A, all groups had different concentrations of IL-8 at day 3. NC group stands out from all groups, besides PT group, by significantly resulting in a higher concentration of IL-8 compared with groups ND, NT, PD and PC.

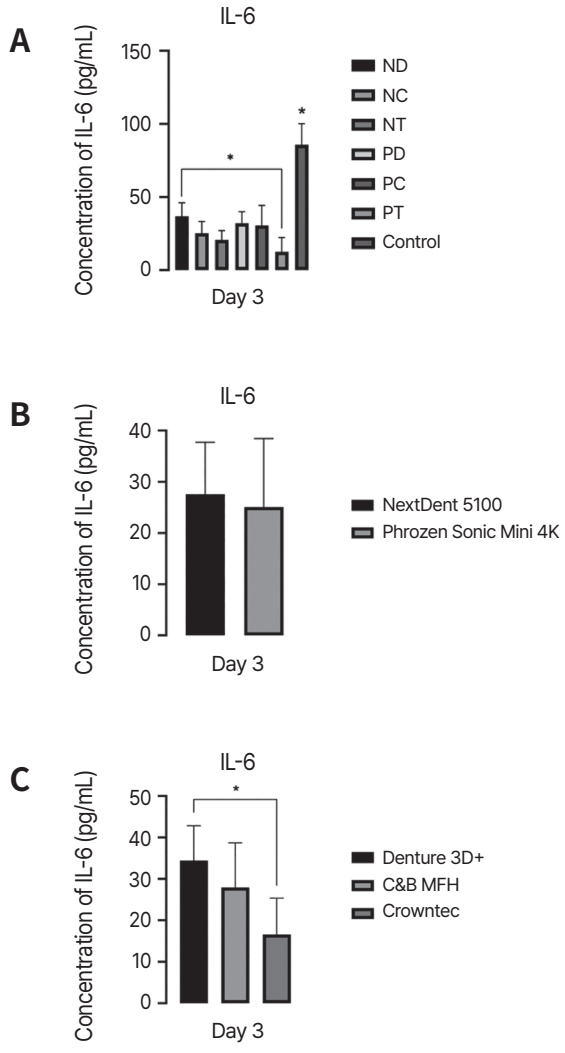

Fig. 2. Bar chart depicting mean IL-6 concentrations in cell culture media (pg/mL), for groups ND, NC, NT, PD, PC, PT and negative control after 3 days of culture (n = 4).

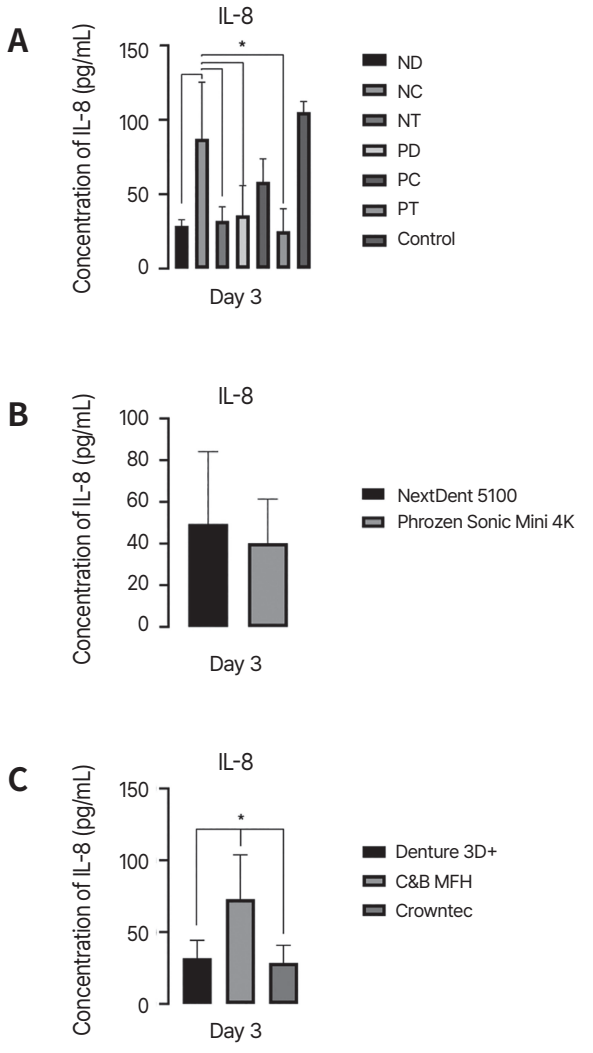

Fig. 3. Bar chart depicting mean IL-8 concentrations in cell culture media (pg/mL), for groups ND, NC, NT, PD, PC, PT and negative control after 3 days of culture (n = 4).

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Similarly to IL-6, no differences were found between the NextDent 5100 and the Phrozen Sonic Mini 4K printers (as seen in Fig. 3B) in the concentration of IL-8.

When comparing the three resins, C&B MFH resin resulted in a significantly higher concentration of IL-8, when compared to Denture 3D+ (P = .004) and Crowntec (P = .002), as seen in Fig. 3C.

FEG-SEM images were obtained after 1 and 7 days of culture, with successive magnification (between ×100 and ×2000). All specimens observed attached fibroblasts, as seen in Fig. 4, but with differences in morphology and distribution. PD group presented a flatter anatomy, with fewer cellular extensions and narrower cell bodies at day 1 of culture and PT group presented a higher distribution and wider adhesion of fibroblasts.

Crystalized precipitates, probably derived from phosphate-buffered saline, were apparent in all samples preventing further cell discrimination and image analysis.

Similarly, fluorescence microscopy images were obtained after 1 and 7 days of culture, and all specimens observed adherent cells, but without a true scattering of cell bodies characteristic of fibroblasts. At day 7, for groups PC and PT, fibroblasts exhibited a spindle-like appearance, accompanied with a higher density of cells, as seen in Fig. 5.

Regarding surface roughness, according to the statistical analysis, no significant differences were found among the different groups (P = .061), as seen in Table 3, which lists the R_a mean and SD values in μm.

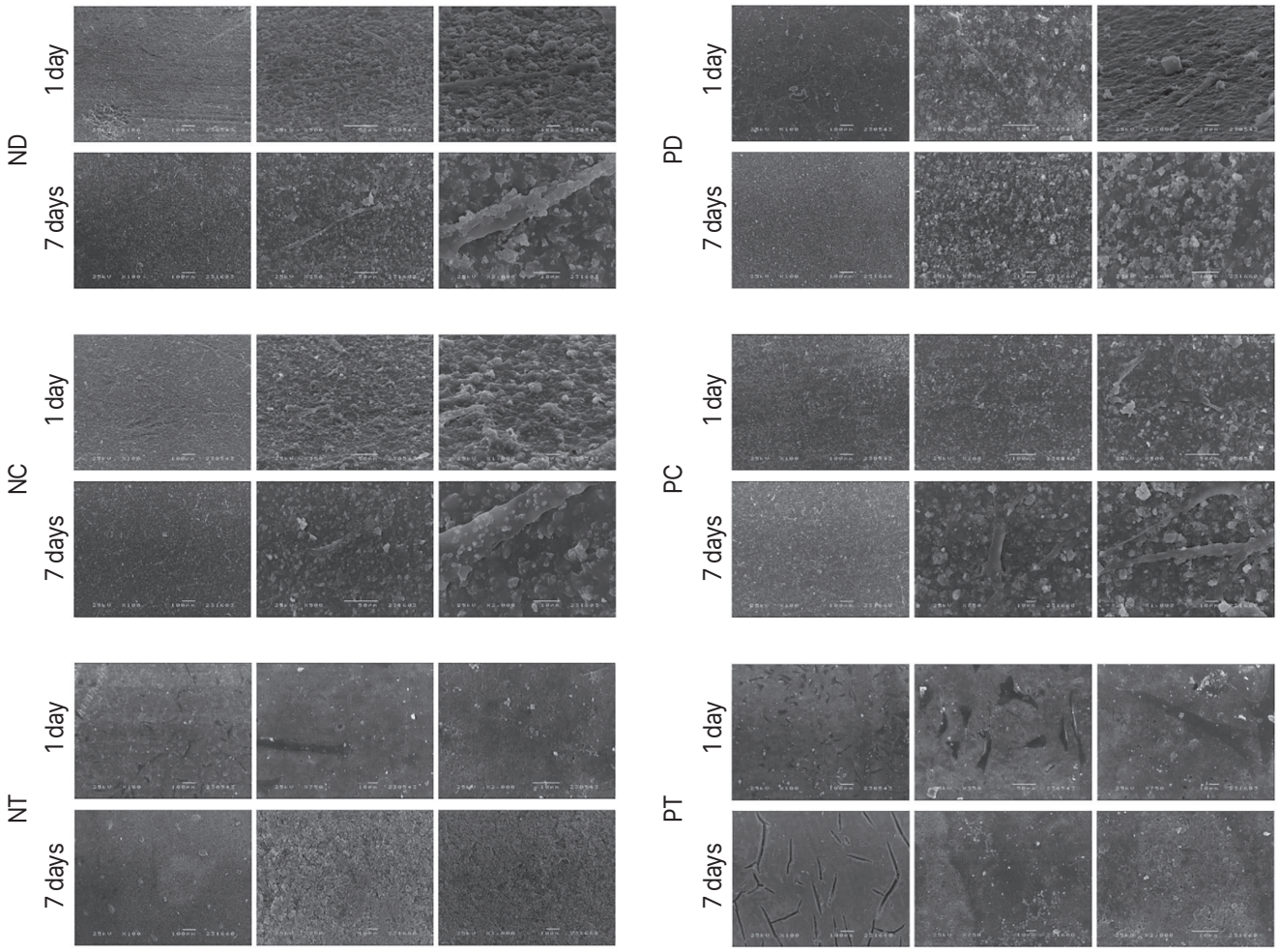

Fig. 4. FEG-SEM images of specimens cultured with HGF-hTERT of groups ND, NC, NT, PD, PC and PT at 1 and 7 days of culture.

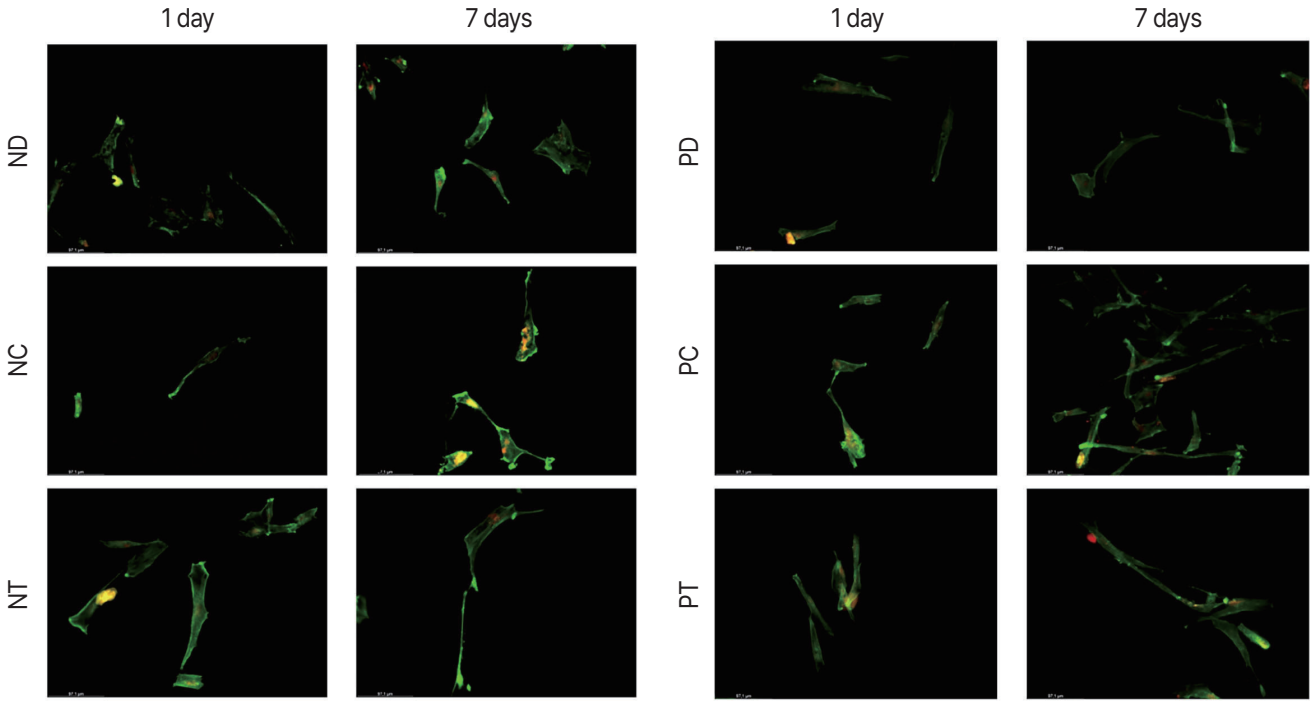

Fig. 5. Fluorescence images of specimens cultured with HGF-hTERT of groups ND, NC, NT, PD, PC and PT at 1 day and 7 days of culture.

Table 3. Surface roughness values for each group, presented with the mean Ra ± SD in micrometers (μm) for Groups ND, NC, NT, PD, PC and PT

Group	Mean R_a (μm)	Standard deviation (SD) (μm)
ND[1]	0.723167	0.1663771
NC[2]	0.710750	0.1825256
NT[3]	0.432000	0.0772528
PD[4]	0.779125	0.1555883
PC[5]	0.807833	0.1497250
PT[6]	0.479375	0.1663778

DISCUSSION

AM is considered a comparable alternative to SM, but there is a lack of studies about cellular behavior and biocompatibility of different 3D printers and dental printable materials. Therefore, this study was designed to evaluate the behavior of fibroblasts in 3D printed resin surfaces, using two different printers and three different resins for specific purposes implying close contact with gingival tissues: removable prosthesis base and fixed provisional restorations.

We performed a direct contact *in vitro* assay using an immortalized gingival fibroblast cell line to evaluate the potential cytotoxic and inflammatory effects of these materials. According to the cellular viability results, all samples resulted in cellular proliferation, but with a significant difference among all groups and the control group, as expected and as observed in similar studies using resin material discs, such as the 2021 study by Srinivasan *et al*.[21] This effect may be related to the fact that discs from each material were used in the bottom of the wells, and a direct contact assay was performed, thus creating less favorable physical conditions for cell attachment and proliferation as compared to control. However, similar conditions among all study groups were obtained. Therefore, the use of a control group serves for assay validation rather than for direct comparisons and percent viability calculation.

When comparing the effect of the different printers

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on cellular viability, no significant differences were found between the NextDent 5100 printer and the Phrozen Sonic Mini 4K printer, at any point in time, allowing for the acceptance of the primary null hypothesis. This conveys that, in terms of biocompatibility, no differences were observed between the printer recommended by the resin manufacturer and a third-party printer, which is not specifically designed for the production of dental medical devices, such as the Phrozen Sonic Mini 4K used in this study.

However, when comparing the effect of the different resins on cellular viability, a significant difference was found at day 1, day 3 and day 7, thus allowing the rejection of the secondary null hypothesis. Despite this, there were significant differences between the Denture 3D+ resin and the Crowntec resin and between the Denture 3D+ resin and the C&B MFH resin, with no significant difference found between the C&B MFH resin and Crowntec resin, which are both meant to be used as temporary crown materials with a limited surface area of contact with gingival tissues (with Crowntec being suitable for permanent crowns as well), as opposed to denture base materials, which are in a more permanent and intimate contact with the oral mucosa.

Considering the effect of each type of resin, overall, the Denture 3D+ resin had the lowest viability results, regardless of the printing method used. Both the PD group and ND group used the Denture3D+ resin. The PT group, which was produced by the Phrozen Sonic Mini 4K printer and using the Crowntec resin, has a significant increase in cellular viability, when compared to the PD group, which was also produced via the Phrozen Sonic Mini 4K printer, and ND group, which was produced using the NextDent 5100 printer.

The ND group had a significant decrease in cellular viability, when compared to all groups, which indicated that the Denture3D+ resin was inferior in terms of biocompatibility to the resins used to manufacture temporary crowns (as is the case of C&B MFH resin) or permanent crowns (as is the case of Crowntec resin). This was not expected, given the fact that Denture3D+ resin is intended to manufacture dentures, which are classified as long-term use medical devices. For this reason, it is important to understand the reason behind the lower biocompatibility values, such as color pigments or other additives.

Supporting the results from this study, Bürgers *et al*. evaluated the cytotoxicity of 3D printed resins used in occlusal splints, which are chemically similar to denture resins, and found that the chemical composition of the resin was more relevant for cytotoxicity, rather than the printing technology. The authors attribute this to the different type of monomers, additives and initiators present in the resins, which can affect biocompatibility. Wedekind *et al*. concluded that residual monomers and additives that eluded from 3D printed materials resulted in cytotoxicity for human gingival fibroblasts and could cause allergies and cross-reactions.[27,28] Guerrero-Gironés *et al*.[29] found that the NextDent Ortho Rigid resin had similar cellular behavior to conventional resins, supporting the use for occlusal splints, which are also used long-term. Frasheri *et al*.[30] concluded that 3D printed materials, which included the NextDent C&B MFH resin, affected cell proliferation and induced more unfavorable effects on gingival keratinocytes.

When comparing the composition of the resins used in this study, all are considered class IIa biocompatibility materials, which are materials to be used for longer than transient contact, such as restorative materials, which is in conformity with what was expected.[31] All of the resins had a different composition, with all ingredients of Denture3D+ being in the C&B MFH resin, but in different proportions. Interestingly, the first ingredient listed in the C&B MFH resin is related to skin sensitivity and is also the second ingredient listed in the Denture3D+ resin. Both resins include TiO_2 in the list of ingredients, but at a very low percentage (less than 0.1% w/w), which is said to improve antimicrobial properties.[10] The Crowntec resin does not specify the proportions of all ingredients and which initiators are used, unlike the previous two resins. All ingredients mentioned in the Safety Data Sheet of Crowntec resin are different from the Denture3D+ and the C&B MFH resin.

Due to the manufacturing method, after the structure has been printed by the 3D printer, an additional polymerization step is required, which can lead to increased polymerization shrinkage and deformation (when removing the structure from the build platform). For this reason, when producing the spec-

imens, this additional polymerization step was performed, following manufacturer instructions. In addition, it is necessary to remove the surface layer of unpolymerized resin, using isopropyl alcohol, in order to reduce the amount of residual monomer and improve biocompatibility.[9,10,21,32] This protocol was also followed in this study, but the same protocol was applied in all samples and therefore the isolated effect of this step was not assessed as it was not an objective of the present work.

In the present study, no polishing was performed in the specimens, since the intaglio surface of the denture isn't usually polished, as it may affect adaptation to the soft tissues. The lack of polishing is said to influence the biocompatibility of materials, since the removal of the outermost layer can remove potential leachable substances. These leachable substances were found to be ovo-toxic by Rogers *et al*.[33] A study by Bieger *et al*. found that the printed specimens that were only washed in isopropyl alcohol and cured (similarly to the present study) had a severe cytotoxic effect on human gingival fibroblasts, with the polished specimens being similar to conventional and milled specimens. For this, the authors suggest that the printed materials should only be used short-term.[24] Therefore, given our results, we can determine that perhaps resins such as the Denture 3D+ resin shouldn't be considered for long-term use.

Gingival fibroblasts play an important role in tissue homeostasis by production and modulation of immune responses through cytokine secretion.[34] Cytokines have pro-inflmmatory functions, such as IL-6 and IL-8, or anti-inflammatory functions, such as IL-10 and TGF-β. Il-6 stimulates antibody production and matrix-metalloproteinases, whose function is to destroy collagen fibers. IL-8 is a a major mediator of the inflammatory response and acts as a chemoattractant, inducing a neutrophil migration.[26,35,36]

When comparing the concentration of inflammatory mediators, such as IL-6 and IL-8, there is a significant difference among the groups, with the ND group resulting in a higher concentration when compared to PT group for IL-6. For IL-8, the NC group significantly resulted in a higher concentration of IL-8 when compared to ND, NT, PD and PT groups. Interestingly, both groups were produced using the NextDent 5100 printer. A possible explanation for these findings may be the different chemical composition of the three resins used in this study. As listed in the Safety Data Sheet, both NextDent C&B MFH and NextDent Denture 3D+ resins have a 1-5% w/w of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO), which is the photo initiator used in all three resins. In the Crowntec resin, this component only makes for 0.1 - 1% w/w of the mixture. The unreacted TPO has been proven to exhibit genotoxic and cytotoxic effects and has also been proven to be more cytotoxic than other photo initiators, such as camphorquinone. This may explain why NT and PT groups had the lowest mean values of IL-6 and IL-8. Additionally, since photo initiators initiate polymerization, if part remains unreacted, a lower degree of conversion is expected, which leads to a higher concentration residual monomer.[36-38] For this reason, it may indicate that the NextDent 5100 printer or a specific equipment-related variable leads to a lower degree of conversion.

This study can conclude that C&B MFH resin leads to similar cellular proliferation as Crowntec resin, whilst being a more cost-effective option.

Since surface roughness is a significant variable affecting cellular behavior, it was evaluated in a representative sample of specimens from each group. No significant differences were found among the groups, leading to the conclusion that the manufacturer's recommended printer and the third-party printer led to similar roughness properties. There was a difference in surface roughness in the groups using the Crowntec resin, but it failed to be statistically significant. A study by Srinivasan *et al*. in 2021 also evaluated the surface roughness of 3D printed specimens, using the NextDent Denture 3D+ resin and printing with a manufacturer recommended printer (Rapid Shape D30) and a third-party printer (Form 2). The authors concluded that the specimens printed with the Rapid Shape D30 printer was significantly smoother than the specimens printed with Form 2 printer.[21] Given that our study compared different printers from the ones used in the study by Srinivasan *et al*., no comparison can be established, and we can only conclude that there were no significant differences in the printers used in our study. Roughness strongly influences fibroblast behavior in resin materials as stated by

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many studies, with polished, lower roughness surfaces leading to higher cell adhesion and viability. Based on this, we placed the hypothesis that different resin types or printers could lead to different roughness values and lead to distinct cell behaviors, but we did not find differences in roughness, rejecting this hypothesis. So, the observed differences in cell behavior may be related to other material properties than roughness, such as the chemical composition of the materials, their conversion rate, and amount of residual monomer or other physical properties such as wettability or solubility.[39-41]

To our knowledge, this is the first time that the biological effects of 3D printed dental resins produced using a manufacturer recommended 3D printer versus a third-party 3D printer were studied. Three different resins for distinct purposes in dental medical device production were used and printed in two different 3D printers using equivalent production and post-production parameters. This study brings important data demonstrating that from a biological point of view, when the same FDA-approved dental resins for 3D printing are used, general consumer PLA printers perform as well as high-end professional dental used 3D printers. These results consider cell viability, inflammatory marker secretion and interaction with materials, using a representative cell line from the oral mucosa.

A limitation of this study is that no evaluation of mechanical properties was performed. However, surface roughness was evaluated in all samples and equivalent values were obtained, demonstrating that the observed differences in cellular behavior are not related to roughness, but rather to other surface properties, potentially related to surface chemistry. Similarly, in 2022, a study by Al-Dwairi *et al*.[42] found no statistically significant differences among the surface roughnesses of specimens produced with three different 3D printing resins, which included the NextDent Denture 3D+ resin.

Similarly, in a comparison among printers and resins considering the accuracy performance, a critical parameter in the clinical decision for customized dental medical device production was not performed, since it fell out of the scope of this study. However, a study by Atria *et al*.[43] in 2022 compared the mechanical characteristics between the Crowntec and NextDent C&B MFH resins and came to the conclusion that the Crowntec specimens had similar values for characteristic stress to conventional resin materials and PMMA milled blocks, corroborating the indication for long-term use. Another potential concern was the inability to access the complete list of ingredients for all resins, especially the Crowntec resin, as to research the color pigments used, for example, and their relation to cellular biocompatibility. Finally, this was an *in vitro* study and using cells in culture might not be able to replicate the complex conditions and interactions of cells in a living organism, limiting the value of these *in vitro* data to predict *in vivo* behavior.

Due to the limitations of the present study, our data must be considered preliminary. Future studies should research the *in vitro* cellular behavior of human fibroblasts, as well as mechanical effects, of different post-processing protocols that may influence the amount of residual monomer present in the resin, which should also be measured. Also, the findings of this study should be confirmed using more complex testing models, namely 3D engineered oral mucosa models, and in the long term, *in vivo* models.

CONCLUSION

Based on the findings of the present study, the use of different 3D printers with equivalent parameterization does not influence the *in vitro* cellular proliferation of human fibroblasts. However, the use of different 3D printing resins moderately influences the *in vitro* cellular behavior of human fibroblasts, with Denture 3D+ resin resulting in significantly lower biocompatibility, when compared to C&B MFH and Crowntec resins. The use of different 3D printers or resins does not significantly influence surface roughness.

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