Universidade de Lisboa
Faculdade de Medicina Dentária

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Dissertação
Mestrado Integrado em Medicina Dentária
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ACKNOWLEDGMENTS

À Professora Doutora Joana Marques, minha orientadora, por todas as horas e paciência que teve, não só durante este projeto, mas também durante as várias Unidades Curriculares que leciona. Obrigada por todos os conhecimentos e sabedoria que me transmitiu, e pelo seu empenho e bem-estar, que sempre me trouxe tranquilidade, mesmo nos momentos mais difíceis.

À Professora Doutora Mariana Brito da Cruz, coorientadora deste projeto, por todo o conhecimento partilhado e pela sua exigência, determinação e boa-disposição. Obrigada por sempre me incentivar a fazer mais e melhor.

Ao Professor Doutor António Mata, Investigador Principal do GIBBO, pela oportunidade de fazer parte deste grupo de investigação, que não só me abriu as portas para a área da Investigação, mas também me ajudou a lidar melhor com a frustração e gestão de tempo.

Ao Professor Doutor Duarte Marques, pelo seu exemplo de dedicação, sendo que sempre demonstrou que com vontade e esforço, tudo é possível.

Ao Professor Doutor João Silveira, que incutiu desde cedo o gosto pela investigação, sempre com a melhor vontade.

Ao Professor Doutor João Paulo Martins, Professor Doutor João Carlos Roque e Rodrigo Malheiro, por toda a ajuda e disponibilidade prestada ao longo deste projeto.

Ao GIBBO por sempre me fazerem sentir integrada e por todos os conhecimentos que adquiri. Um agradecimento especial à Dra. Ana Marques, à Dra. Neusa Silva e à Rafaela por todo o apoio, conhecimento e carinho que me deram ao longo destes anos.

Aos meus pais, por todo o amor, carinho e apoio que me deram. Por todos os jantares que me levaram ao escritório para continuar a estudar no 3º ano. Por me incentivarem sempre a fazer mais e melhor, sem desistir. Por estarem lá, sempre que precisava. Por serem exemplos de determinação e sucesso, mas também de união e amizade. Nunca teria conseguido terminar sem vocês.

Aos meus avôs e avó Maria, que já partiram, mas que sei que sempre olharam por mim e irão continuar. Levo-vos todos os dias no meu coração e sei que estariam orgulhosos do meu percurso. À minha avó Fernanda, pelo exemplo de superação que sempre incutiu em mim. Obrigada por toda a companhia, amizade e amor. Que continues a ser a avó mais moderna que conheço.

Aos meus tios, por todo o apoio e encorajamento. Fico à espera da clínica patrocinada e prometo que para vocês é grátis. À Madalena, por toda a amizade e carinho. Sei que serás a melhor no que escolheres ser, seja pintora ou astronauta, e que continuarás a ser a menina mais querida e linda de Portugal. Fico à espera pela nossa primeira saída à noite. À Júlia e Miguel, por todos os momentos de ternura e pelas as vossas gargalhadas, que sempre me trouxeram felicidade e alegria, mesmo nos momentos mais exigentes.

Ao Exército, por me terem acompanhado nesta longa viagem e por tornarem todos os momentos especiais. Obrigada por me fazerem uma pessoa mais divertida e feliz. Que continuemos de mãos dadas na próxima etapa. Adoro-vos muito.

ABSTRACT

Objectives: The aim of this study was to evaluate the influence of different 3D dental printers and resins on human gingival fibroblasts behaviour.

Materials and Methods: Three resins from NextDent® (Denture 3D+®, C&B MFH® and Crowntec®) were used to produce disc-shaped specimens on NextDent® 5100 and Phrozen® Sonic Mini 4K printers, using equivalent parameters in a total of 6 groups (N=20), with groups PD, PC and PT corresponding to Denture 3D+®, C&B MFH® and Crowntec® printed on Phrozen printer, respectively and ND, NC and NT corresponding to Denture 3D+®, C&B MFH® and Crowntec® printed on NexDent printer, respectively . Human gingival fibroblasts were cultured on specimens and biocompatibility evaluated at 1,3 and 7 days. IL-6 and IL-8 concentrations were evaluated at 3 days of culture using ELISA. Surface roughness was evaluated by a contact profilometer. SEM and fluorescence micrographs were analyzed at 1 and 7 days of growth. Statistical analyses were performed using SPSS 28.0 version and mean differences were tested using ANOVA and *post-hoc* Tukey tests ($p< 0.05$).

Results: There was an increase in cellular growth after 7 days in culture in group PC and in group PT when compared to group PD (p=0,028 and p=0,203, respectively). ND group resulted in higher concentration of IL-6 when compared to PT group, and NC resulted in higher concentration of IL-8 when compared to ND, NT, PD and PT groups. No significant differences were found between groups regarding surface roughness.

Conclusions: Within the limitations of the present study, NextDent® 5100 and Phrozen® Sonic Mini 4K performed similarly considering cell responses. The use of different 3D-printing resins influences in-vitro cellular behaviour of human fibroblasts. Surface roughness did not seem to be influenced using different printers or resins, if equivalent parameters are used.

Key-words: dentures; Computer-Aided design; printing, three dimensional; materials testing; resins, synthetic; surface properties

Clinical significance: 3D printing has gained popularity in recent times, including the production of complete dentures. The results of this study suggest that the use of different

printers does not influence biocompatility, when using equivalent parameters. However, different resins influenced cellular behaviour, in particular Denture 3D+® resin. For this reason, we suggest that perhaps resins such as Denture 3D+® resin shouldn't be used long-term.

RESUMO

A técnica aditiva, também conhecida por impressão 3D, tem ganho popularidade nos últimos anos para produção de próteses removíveis totais, sendo considerada uma alternativa comparável à técnica subtrativa e permite uma relação custo-benefício mais vantajosa, ao requerer equipamentos menos dispendiosos e permitir a confeção de vários objetos simultaneamente. No entanto, está descrita na literatura uma escassez de estudos de biocompatibilidade referentes aos materiais utilizados e aos diferentes métodos de produção. Adicionalmente, apenas existe um estudo que compara a resposta celular a resinas impressas tridimensionalmente com uma impressora recomendada pelo fabricante e uma impressora *third-party* dispendiosa.

Este estudo pretendeu avaliar as respostas celulares às resinas impressas em 3D, utilizando uma impressora recomendada pelo fabricante e uma impressora *third-party* de menor custo. Tendo em conta que as próteses removíveis estão em íntimo contato com a mucosa, a cultura celular de eleição foram os fibroblastos gengivais humanos, já que são o tipo celular dominante no tecido conjuntivo.

O objetivo deste estudo foi avaliar a influência de diferentes impressoras 3D e resinas no comportamento dos fibroblastos. Como hipótese nula primária, consideramos que o uso de diferentes impressoras 3D com parametrização equivalente não influencia o comportamento celular in vitro de fibroblastos humanos. A hipótese nula secundária considerou que o uso de diferentes resinas não influenciaria o comportamento celular *in vitro* de fibroblastos humanos.

Desta forma, foram selecionadas três resinas para impressão 3D da marca NextDent®, Denture 3D+ na cor Translucent Pink (NextDent®, Soesterberg, Holanda), C&B MFH na cor N1 (NextDent®, Zeist, Holanda) e Crowntec na cor A2 (SAREMCO®, Rebstein, Suíça). Foram selecionadas duas impressoras 3D NextDent 5100 (3D Systems®, Carolina do Sul, Estados Unidos da América) e Phrozen Sonic Mini 4K (Phrozen®, Hsinchu, Taiwan). Durante a utilização das impressoras foram usados parâmetros equivalentes, utilizando uma espessura de 50μm por camada e orientação vertical de impressão. As amostras foram alocadas em 6 grupos, num total de 20 amostras por grupo (N=20).

As amostras foram produzidas em forma de disco, com 8mm de diâmetro e 3mm de espessura. Foram seguidas as indicações do fabricante e, após a impressão, foi realizado o protocolo de pós-polimerização, através da lavagem das amostras com etanol a 96% num banho ultrasónico e processo de cura através da NextDent LC-3D Print Box (NextDent®, Soesterberg, Holanda), durante 30 minutos.

Fibroblastos gengivais humanos imortalizados foram cultivados em Dulbecco's Modified Eagle Medium (Lonza, Visp, Suíça), suplementado com 10% de soro fetal bovino (Biowest, Nuallié, França) e 1% de penicilina/estreptomicina (Lonza, Visp, Suíça).

Foram realizados três ensaios (N=15), sendo que em cada ensaio, cinco amostras de cada grupo foram descontaminadas, colocadas em placas de 48 poços (Corning Inc®, Corning®, Nova Iorque, EUA) e semeadas na densidade de $5x10^3$ células por poço. Um controlo negativo consistindo de células na mesma densidade semeadas em poços vazios foi utilizado em todos os ensaios, de modo a validar os ensaios realizados.

A viabilidade e proliferação celulares foram avaliadas com o reagente Cell-TiterBlue® (Promega®, Madison, EUA), através da redução de resazurina, conforme o protocolo do fabricante. A taxa de conversão do corante azul não fluorescente foi determinada como intensidade de fluorescência em UA após 1, 3 e 7 dias de cultura, através de um leitor de microplacas multimodo (VICTOR NivoTM HH3500, PerkinElmer®, Pontyclun, Reino Unido).

Para quantificar a IL-8 e IL-6 presentes no sobrenadante da cultura celular, foi utilizado o kit Human IL-8 /CXCL8 DuoSet ELISA e o kit Human IL-6 DuoSet ELISA (R&D Systems Inc®, Minneapolis, EUA), de acordo com as instruções do fabricante, sendo medidos às 72 horas de cultura. A absorvância foi medida utilizando um leitor de microplacas multimodo nos comprimentos de onda de 450nm e 540nm e, com base na curva de calibração, foram calculadas as concentrações de IL-8 e IL-6 em pg/mL.

Para avaliar possíveis alterações na morfologia celular foi utilizada a microscopia eletrónica de varrimento (FEG-SEM) e microscopia de fluorescência, sendo que foram descontaminadas 4 amostras de cada grupo, semeadas com HGF-hTERT (nas mesmas condições mencionadas anteriormente) e fixadas com 1 e 7 dias de crescimento.

Para microscopia de fluorescência, inicialmente as amostras foram lavadas com PBS filtrado (VWR®, Radnor, Pensilvânia, EUA) e fixadas com formaldeído (Pancreac Applichem, ITW Reagents Division, Darmstadt, Alemanha) a 4% por dez minutos. As células foram então permeabilizadas com 0,10% Triton X-100® (Sigma-Aldrich, Merck KGaA, Darmstadt, Alemanha) por cinco minutos e foi utilizada uma solução de Faloidina (Phalloidin FITC Reagent – ab235137, Abcam, Waltham, EUA) e uma solução de Iodeto de Propídio (Sigma-Aldrich, Merck KGaA, Darmstadt, Alemanha) para colorir o citoplasma e o núcleo, respetivamente.

Para FEG-SEM, as amostras foram inicialmente lavadas com PBS e fixadas com glutaraldeído (Electron Microscopy Sciences, Hatfield, Reino Unido) a 2,5% durante uma hora.

Foi realizado o processo de desidratação, utilizando concentrações sucessivamente maiores (de 20 a 100%) de etanol (Honeywell Riedel-de Haën, Seelze, Alemanha). No dia de observação a microscópio, um filme de ouro-paládio (Au-Pd) de 80-20% de massa foi colocado sobre as amostras e estas foram observadas com uma voltagem de aceleração de 10kV com sucessivas ampliações.

Para avaliação da rugosidade de superfície, foi realizada perfilometria de contacto, utilizado o Tencor® Alpha-step 200 Profilometer, com uma ponta de 12,5µm de diâmetro, a uma distância de 400µm e com uma força de 11mg.

A análise estatística foi realizada utilizando IBM® SPSS® Statistics 28,0 para macOs (SPSS, Chicago, EUA) e GraphPad Prism 9 para macOs (GraphPad Software, Inc. San Diego CA, EUA). A distribuição de normalidade foi avaliada para todas as amostras utilizando o teste de Kolgromov-Smirnov. A comparação entre os grupos foi realizada com base na análise de variância (ANOVA) de uma via, coadjuvante aos testes *post-hoc* de Tukey. O nível de significância foi definido como $p<0,05$ e todos os resultados foram apresentados como média ± desvio padrão (DP).

Foi observado um aumento significativo do crescimento celular após 7 dias em cultura no grupo PC (impresso com a resina C&B MFH®, pela impressora Phrozen® Sonic Mini 4K) e no grupo PT (impresso com a resina Crowntec®, pela impressora Phrozen® Sonic Mini 4K), quando comparado ao grupo PD (impresso com a resina Denture 3D+®, pela impressora Phrozen® Sonic Mini 4K) ($p=0,028$ e $p=0,203$, respectivamente).

Não foram observadas diferenças significativas, em qualquer momento, ao comparar grupos produzidos com a impressora NextDent® 5100 e a impressora Phrozen® Sonic Mini 4K.

Ao comparar a viabilidade celular por resina, foi verificada uma diminuição significativa da viabilidade da resina Denture 3D+®, face à resina Crowntec® após 1 dia ($p<0,001$). Ao terceiro e sétimo dia, existiu uma diminuição significativa da viabilidade na resina Denture 3D+®, comparativamente à resina C&B MFH® ($p=0,008$ no dia 3 e $p=0,002$ no dia sete) e Crowntec® ($p<0,001$ no dia 3 e 7). Não foi verificada uma diferença significativa entre as resinas C&B MFH® e Crowntec®.

Relativamente à resposta inflamatória, foi verificada uma maior concentração de IL-6 do grupo ND (impresso com a resina Denture 3D+®, pela impressora NextDent® 5100) face ao grupo PT. Foi também verificada uma maior concentração de IL-8 do grupo NC (impresso

com a resina C&B MFH®, pela impressora NextDent® 5100) face aos grupos ND, NT (impresso com Crowntec®, pela impressora NextDent® 5100), PD e PT.

Relativamente à morfologia celular, foi observado que grupo PT apresentava uma maior distribuição e adesão de fibroblastos e que os mesmos apresentavam uma aparência fusiforme. Os fibroblastos existentes nas amostras do grupo PD apresentavam uma aparência mais achatada e estreita, com menos prolongamentos celulares.

Relativamente à rugosidade de superfície, não foram encontradas diferenças significativas entre os grupos.

Tendo em conta os resultados deste estudo, é possível concluir que as impressoras NextDent® 5100 e Phrozen® Sonic Mini 4K apresentaram um desempenho semelhante, considerando as respostas celulares. Desta forma, a hipótese primária nula foi aceite.

É possível também concluir que o uso de diferentes resinas influencia o comportamento celular *in-vitro* de fibroblastos humanos, evidenciado pelas diferenças significativas encontradas nos 3 momentos de viabilidade celular. Desta forma, a hipótese secundária nula foi rejeitada.

A resina Denture 3D+® apresentou o pior comportamento celular, sendo que são necessários mais estudos, de modo a determinar se esta resina é compatível com o uso a longo prazo, necessário para a utilização de uma prótese removível. Podemos concluir que a resina C&B MFH® promove uma proliferação celular semelhante à resina Crowntec®, sendo uma opção menos dispendiosa.

Adicionalmente, a rugosidade da superfície não parece ser influenciada pelo uso de diferentes impressoras ou resinas, se forem utilizados parâmetros equivalentes .

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LIST OF ABBREVIATIONS

ºC – Degree Celsius

% - Percentage

p – *p*-value

µl - Microlitre

µm – Micrometer

3D – Three-dimensional

AM – Additive manufacturing

ANOVA – Analysis of variance

AU – Arbitrary fluorescence units

cm^2 – Square centimeter

CAD-CAM – Computer-aided design and computer-aided manufacturing

CAM – Computer-aided manufacturing

CD – Complete dentures

DIGITECH – Laboratório Tecnologias Digitais

DLP – Digital light processing

DMEM – Dulbecco's Modified Eagle's Medium

ELISA – Enzyme-linked immunosorbent essay

FBS - Fetal bovine serum

FDA – Food and Drug Admnistration

FEG-SEM – Field Emission Gun Scanning Electron Microscopy

FMDUL – Faculdade de Medicina Dentária da Universidade de Lisboa

HGF-hTERT – Human Gingival Fibroblast immortalized by human telomerase reverse transcriptase gene

IL - Interleukin

INESC MN - Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias

IOS – Intraoral scanner

KA - KiloAngstrom

mm – Millimetre

nm – Nanometre

PBS – Phosphate-buffered saline

pg/mL – Picogram per millilitre

R_a – Surface roughness

® - Registered trademark

SD – Standard deviation

SLA – Stereolithography

SM – Subtractive manufacturing

TiO^2 – Titanium dioxide

TPO – diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide

w/w – Weight for weight

ZrO^2 – Zirconium dioxide

INTRODUCTION

Computer-aided design and computer-aided manufacturing (CAD-CAM) systems were first introduced in Dentistry in the 1980s and 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]. In CAD-CAM, an image is first captured (using an intra or extra-oral scanner), the object is then designed in a specific software (CAD) and is produced through computer-aided manufacturing (CAM)[1–4].

Nowadays, these techniques effectively reduce chair time and allow the storage of information through a digital file, for further usage[5–7]. There are two main different CAM approaches: subtractive manufacturing (SM) and additive manufacturing (AM)[3,4]

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

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]. There are 7 different types of production using the additive technique, the most used in Dentistry being stereolithography (SLA) and digital light processing (DLP). In both techniques, the build platform is immersed in liquid resin, where a light source draws a cross-section of the object to form a layer. This layer is polymerized by UV light and the process is repeated until the structure is complete. The difference between SLA and DLP is the light source, SLA uses a laser and in DLP the image is created through microscopic mirrors (greater number of mirrors correspond to higher image resolution)[4,15].

Various factors influence 3D-printing, such as printing orientation. 3 axes compose a 3D-printer, two horizontal axes (X and Y) and one vertical axis (Z), which is associated with accuracy, due to less light reflection. According to Li *et al.* a 45º build angle resulted in the higher surface roughness and surface energy. Additionally, this angle was associated with higher error rates in the same study. Contrarily, a 90º printing orientation had the lowest error rates and

highest flexural strength, leading, however to higher microbial adhesion. These results were attributed to the higher surface roughness generated by the 45º build angle [8,15].

After the structure has been printed, an additional polymerization step is required, which can lead to increased polymerization shrinkage and deformation (when removing the structure from the build platform). In addition, it is necessary to remove the surface layer of unpolymerized resin, using ethanol, in order to reduce the amount of residual monomer and improve biocompatibility[9,10,16,17].

One interesting possibility in additive manufacturing is to modify 3D printing resins, by incorporating certain ingredients with the potential to improve the physical or mechanical properties of the final structure. Teixeira *et al*. states that incorporating titanium dioxide (TiO^2) nanoparticles coupled with a silane agent improves antimicrobial properties (when added in concentrations of 1 or 2%)[10]. A study by Alshaikh *et al.* concluded that zirconium dioxide (ZrO^2) nanoparticles on 3D-printed denture base material improved flexural and impact strength, without increasing surface roughness significantly. Furthermore, ZrO^2 is biocompatible and has antibacterial and antifungal properties[18]. While several studies have proposed the modification of the base material to improve its properties and showed promising results, to date there is no consensus of the best material and the most efficient combination of ingredients, concentration or technique approach. Additionally, there are insufficient studies on the repercussion of these modifications on mechanical resistance.

Digital technologies are currently being used for the production of temporary crowns. Temporary crowns and bridges are fixed protheses which are designed to be used for a short amount of time, which are then are substituted by a permanent alternative. Depending on the amount of time, different materials and techniques can be chosen, such as direct, indirect techniques (where CAD-CAM can be used) or a combination of both. Temporary rehabilitations are extremely important for tooth protection, soft tissue adaptation and esthetics[19,20]. A systematic review and meta-analysis by Jain *et al.* concluded that 3D-printed temporary crowns can be used as an alternative to conventional and milled crowns, despite having worst physical properties (such as colour stability and water sorption) and lower resilience and toughness. However, fracture and flexural strength, elastic modulus and wear resistance were superior in 3D-printed crowns[21].

Regarding complete dentures (CDs) [14]the intaglio surface evaluation is the key aspect on soft tissues adaptation. 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[22–24]. CAD-CAM technologies enable the possibility to produce a try-in denture before the definitive one, which allows for the evaluation of jaw relation, aesthetics and function. Since 3D-printing has less waste of material and is more cost-effective, Herpel *et al.* considered an adequate choice for the production of try-in dentures and comparable to conventional techniques[25]. 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[26–29].

There is a lack of studies regarding 3D-printed materials and printers behaviour on the oral cavity, as mentioned in articles from Kalberer *el at.* in 2018, Lemos *et al.*, Vilela-Teixeira *et al.* and Schweiger *et al.* in 2021[9,10,30,31]. A systematic review from Srinivasan *et al.* which evaluated various parameters of CAD-CAM dentures, however related with biocompatibility only one study of 3D denture base material, by Müller *et al.* in 2019, was included, which evaluated milled and 3D-printed materials and found no difference between the groups[32]. A biocompatible assay from Srinivasan *et al.* was conducted in 2021 which concluded that milled and 3D-printed resins had similar biocompatibility results[16]. One question that may be raised is the influence of the equipment and printing parameters in cell responses to these materials. To our knowledge, only one study compared the cellular responses to 3D-printed dental resins produced using a manufacturer recommended printer and a third-party printer. In this study, the Rapid ShapeD30® (manufacturer-recommended printer) and the Form 2® (third party) printers were compared and found no significant differences between the cellular responses. The third-party printer however is a flagship 3D printer and an expensive equipment [16].

[26–29]The rationale for this study is the fact that while AM is being regarded as a comparable alternative to SM with several advantages, there is a lack of evidence on the biological responses to these materials and how different materials and methods of production affect their biocompatibility. This study intended to evaluate the cellular responses to 3D-

printed dental resins using a manufacturer recommended printer and a less expensive third-party end-user printer. A gingival fibroblast cell line was used based on the fact that dentures are in intimate contact with the mucosa, whose dominant cell type in the connective tissue is gingival fibroblasts[33].

OBJECTIVES

The aim of this study was to evaluate the influence of different 3D printers and resins on fibroblasts behaviour. In order to achieve this goal, the following hypotheses were created:

1. Main hypothesis

H0: The use of different 3D printers with equivalent parameterization does not influence the *in vitro* cellular behaviour of human gingival fibroblasts.

H1: The use of different 3D printers with equivalent parameterization influences the *in vitro* cellular behaviour of human gingival fibroblasts.

2. Secondary hypothesis

H0: The use of different resins does not influence the *in vitro* cellular behaviour of human gingival fibroblasts.

H1: The use of different resins influences the *in vitro* cellular behaviour of human gingival fibroblasts.

MATERIALS AND METHODS

1. Study design

Specimens were produced by the Dental Prosthesis Laboratory - DIGITECH of the Faculdade de Medicina Dentária da Universidade de Lisboa (FMDUL).

Three resins (seen in figure 1) were used in each group: Denture 3D+® in the shade Translucent Pink (NextDent®, Soesterberg, Netherlands), NextDent C&B MFH® in the shade N1 (NextDent®, Zeist, Netherlands) and Crowntec® in the shade A2 (SAREMCO®, Rebstein, Switzerland) produced by two different printers (seen in figures 2 and 3): NextDent 5100 (3D Systems®, South Carolina, United States of America) and Phrozen Sonic Mini 4K (Phrozen®, Hsinchu, Taiwan).

20 disc-shaped specimens were produced for each group with 8mm of diameter and 3mm of thickness, as seen in figure 4. Specimen allocation is seen in table 1.

Figure 1- Photographs of the NextDent resins used in specimen production: Denture 3D+® (colour Translucent Pink, lot number WY213N01, expiration date 20.05.2022), C&B MFH® (colour N1, lot number EX433N03, expiration date 27.10.2023) and Crowntec® (colour A2, lot number E276, expiration date 03.2025), in order.

Figure 2 – Photograph of NextDent 5100® printer used in specimen production for groups ND, NC and NT.

Figure 3 – Photograph of Phrozen Sonic Mini 4K® printer used in specimen production for groups PD, PC and PT.

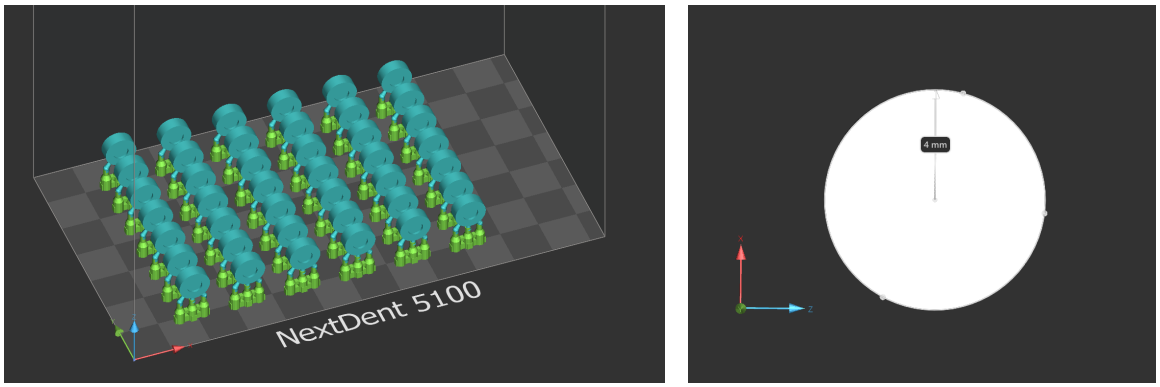

Figure 4 - Representation of specimen design and production (STL file).

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	NextDent 5100	Denture 3D+®
NC		C&B MFH®
NT		Crowntec®
PD	Phrozen Sonic Mini 4K	Denture 3D+®
PC		C&B MFH®
PT		Crowntec®

Specimen production was performed according to the resin manufacturer instructions for both printers, with a thickness of 50µm in each layer and vertical orientation. A closed resin bottle assigned to the test group. Briefly, the bottle was shaken for at least five minutes (to release any material from the bottom of the bottle) and then placed on the NextDent LC-3DMixer roller bench (NextDent®, Soesterberg, The Netherlands) for two and a half hours, seen in figure 5. After this mixing process, the resin was placed in the printer reservoir and the sample was produced, with a thickness of 50µm in each layer and vertical orientation, using the STL file previously created. The same production protocol was used in both equipments NextDent 5100® and Phrozen Sonic Mini 4K®.

After specimen production, the build platforms were removed, and the post-polymerization protocol was performed according to the manufacturer indications for each resin. In short, specimens were washed with 96% ethanol for three minutes in an ultrasonic bath, seen in figure 6. 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 and the curing process was carried out using the NextDent LC-3D Print Box (NextDent®, Soesterberg, The Netherlands) for thirty minutes, as seen in figure 7.

The allocation of specimens for the different essays is depicted in figure 8.

Figure 5 - Photograph of NextDent LC-3DMixer® roller bench used in sample production.

Figure 6 - Photograph of ultrasonic bath used in sample production.

Figure 7 - Photograph of NextDent LC-3D Print Box® used in sample production.

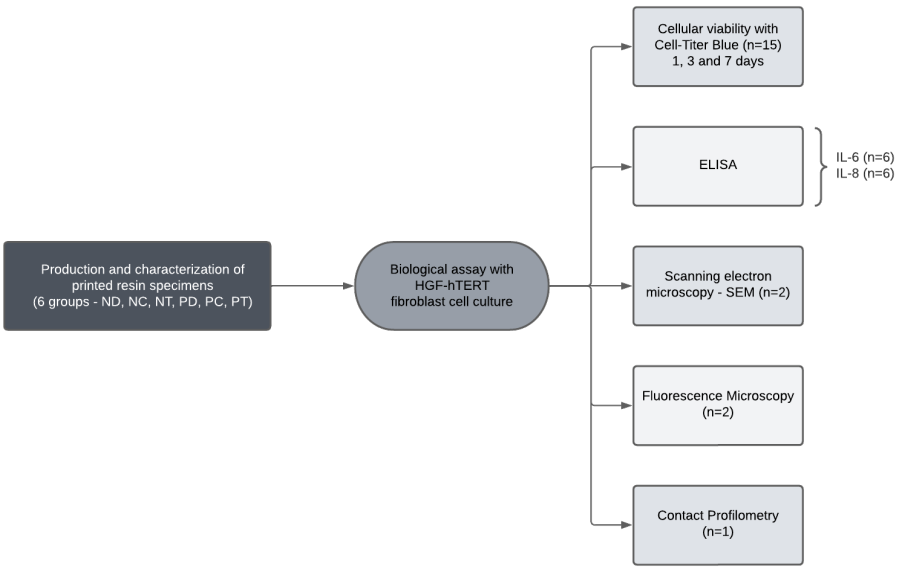

Figure 8 - Study design

2. Cell culture

Cell culture was performed according to aseptic manipulation technique, using a laminar flow chamber (Biobase®, Jinan, China), depicted in figure 9.

Figure 9 - Photograph of horizontal laminar flow chamber used during cell culture.

Human gingival fibroblasts (HGF-hTERT) were cultured in a 75cm^2 culture flask, with 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).

The culture flask was incubated in suitable environmental conditions (5% carbon dioxide, 98% humidity and temperature at 37ºC), using an adaptive incubator (Memmert®, Schwabach, Germany), seen in figure 10.

Figure 10 - Photograph of incubator used during cell culture.

The culture medium was changed one day after seeding and during the growth phase it was changed every two days.

When the cells reached approximately 80% confluence, enzymatic detachment was performed using Trypsin-EDTA (Lonza, Visp, Switzerland) for 5 minutes. Subsequently, cell counts were performed using a Neubauer chamber and Trypan-Blue dye.

For this study, five specimens of each group were decontaminated using ethanol 70% in a ultrasound bath for 30 minutes and placed overnight in UV. These specimens were then placed in a 48-well plate (Corning Inc®, Corning®, New York, USA) and HGFs were seeded at a density of $5x10^3$ cells/per well with 500 µl of culture medium. A negative control consisting of cells at the same density seeded on empty wells was used in all assays. Three cell culture essays were executed to evaluate cellular biocompatibility (total N=15).

3. Biocompatibility essay

Cellular viability and proliferation were evaluated with a Cell-TiterBlue® reagent (Promega®, Madison, USA), by resazurin reduction, 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 NivoTM HH3500, PerkinElmer®, Pontyclun, UK), depicted in figure 11, was used to determine the fluorescence intensity, detected excitation wavelengths of 530/30nm and emission of 595/10nm.

Figure 11 - Photograph of multimode microplate reader used during biocompatibility and interleukin essays.

4. Interleukin essay

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, 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 NivoTM HH3500, PerkinElmer®, Pontyclun, UK) at 450nm and 540nm wavelengths, with the values obtained with the wavelength of 540nm subtracted from those of 450nm, 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.

5. Cellular morphology

The specimens were decontaminated, sterilized, seeded with HGF-hTERT (under the same conditions 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®, Radnor, Pennsylvania, USA) and fixated with formaldehyde (Pancreac Applichem, 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® (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) for five minutes, after this the samples were washed with PBS. To stain the cytoplasm, a solution of Phalloidin (Phalloidin FITC Reagent – ab235137, Abcam, Waltham, USA) was used and, to stain the nucleus, a solution of Propidium Iodide (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Overlapping images were obtained at 400x magnification using a camera attachment to a microscope (Leica DFC3000 G) and corresponding computer program (LAS X 3.6.0.20104, Leica Microsystems, USA).

For 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 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 ultrathin (15nm) 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). SEM images were then obtained in SEM equipment (JEOL JSM-5200LV) available at Faculdade de Ciências da Universidade de Lisboa, with different magnifications (100, 200, 350, 500, 750, 1000 and 2000x). The obtained images were analyzed by two calibrated observers considering cell density, shape and morphology, interaction with surface material, cell spreading and lamellipodia/filopodia formation.

6. Contact profilometry

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 (seen in figure 12) in the INESC MN facilities. The scanning stylus had 12.5μm radius, a distance of 400μm and a tracking force of 11mg. Each specimen was measured in three to four points and the average roughness (R_a) was measured in KA and converted to μm.

Figure 12 – Photograph of contact profilometer used Tencor® Alpha-step 200 Profilometer, available at INESC – MN.

7. Statistical analysis

Statistical analysis was performed using IBM® SPSS® Statistics 28.0 for macOs (SPSS, Chicago, 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 between 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 between groups. Additionally, a one-way repeated measures ANOVA was also performed to compare cellular viability between groups over time. The significance level was defined as $p<0.05$ and all results were presented as a mean ± standard deviation (SD).

RESULTS

1. *Biocompatibility essay*

   According to the results shown in figure 13, all groups presented an increased cellular viability over time.

At day three, significant differences were found between the ND group and the PT group (p=0.013). At day seven, significant differences were found between the PD group and the PC group (p=0.028), and also between the PD group and the PT group (p=0.023).

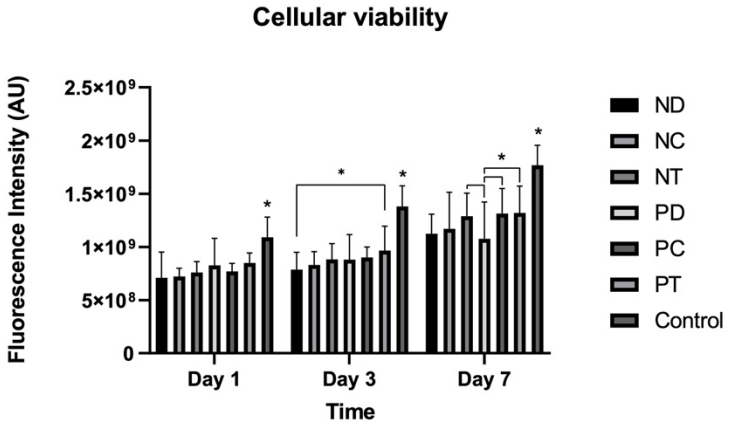

Figure 13 – Bar chart depicting cellular viability results as compound means ± SD in AU from Groups ND, NC, NT, PD, PC, PT and a negative control at 1, 3 and 7 days of culture (N=15). Control group results are shown for essay validation purposes. Error bars represent the SD and a one-way ANOVA with Tukey's post-hoc test was used for comparison between groups. Statistical significance shown: *indicates significant differences between groups (p<0.05).

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 (figure 14).

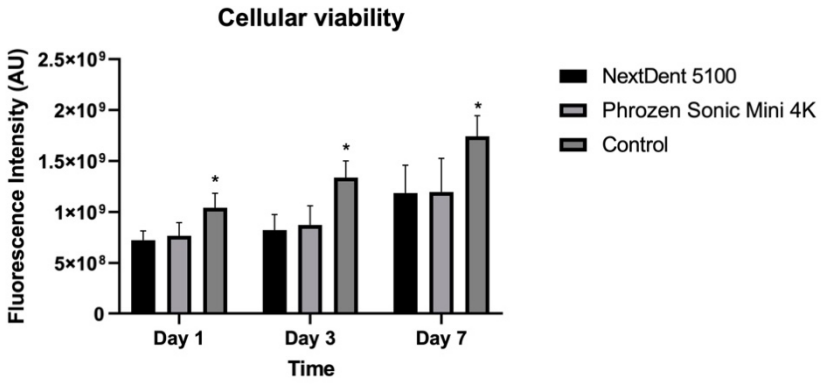

Figure 14 – Bar chart depicting cellular viability results as compound means ± SD in AU from groups printed with the NextDent® 5100 printer, the Phrozen® Sonic Mini 4K printer and a negative control at 1, 3 and 7 days of culture (N=15). Control group results are shown for essay validation puposes. Error bars represent the SD and a one-way ANOVA with Tukey's post-hoc test was used for comparison between groups. *indicates significant differences between groups (p<0.05).

However, significant differences were observed between different resins intended for 3D printing, as seen in figure 15. Specifically, a significant decrease in viability was observed between the Denture 3D+® resin and the Crowntec® resin after 1 day (p<0.001), while at day three and day seven, there is a significant decrease in viability of the Denture 3D+® resin group, comparing to C&B MFH® (p=0.008 at day 3 and p=0.002 at day seven) and Crowntec® resin groups (p<0.001 at day 3 and 7).

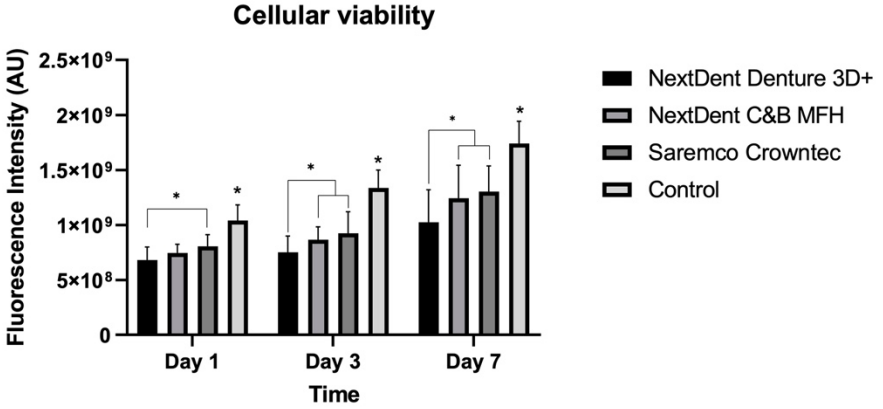

Figure 15 – Bar chart depicting cellular viability results as compound means ± SD in AU from groups printed with the Denture 3D+® resin, C&B MFH® resin, Crowntec® resin and a negative control at 1, 3 and 7 days of culture (N=15). Control group results are shown for essay

validation purposes. Error bars represent the SD and a one-way ANOVA with Tukey's post-hoc test was used for comparison between groups. *indicates significant differences between groups (p<0.05).

2. *Interleukin essay*

According to figure 16, ND group significantly resulted in a higher concentration of IL-6 when compared to NT group.

According to figure 17, NC group significantly resulted in a higher concentration of IL-8 when compared to ND, NT, PD and PT groups.

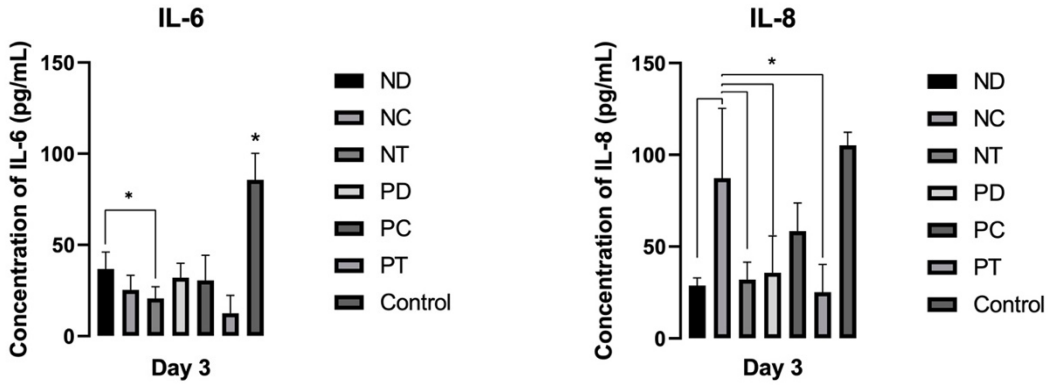

Figure 16 and 17- Bar charts depicting Mean IL-6 (16) and IL-8 (17) concentrations in cell culture media (pg/mL), for groups ND, NC, NT, PD, PC, PT and negative control after 3 days of culture (n=4). Control group results are shown for essay validation puposes. Error bars represent the SD and a one-way ANOVA with Tukey's post-hoc test was used for comparison between groups. *indicates significant differences between groups (p<0.05).

3. *Cellular morphology*

SEM images were obtained after 1 and 7 days of culture, with successive magnification (between 100 and 2000x). Attached fibroblasts were observed in all specimens, as seen in figure 18, however, differences in morphology and density were apparent. PD group presented a flatter anatomy, with fewer cellular extensions and narrower cell bodies at day 1 of culture. At 1 day of culture, 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.

No images from group PC were available due to issues with SEM acquisition.

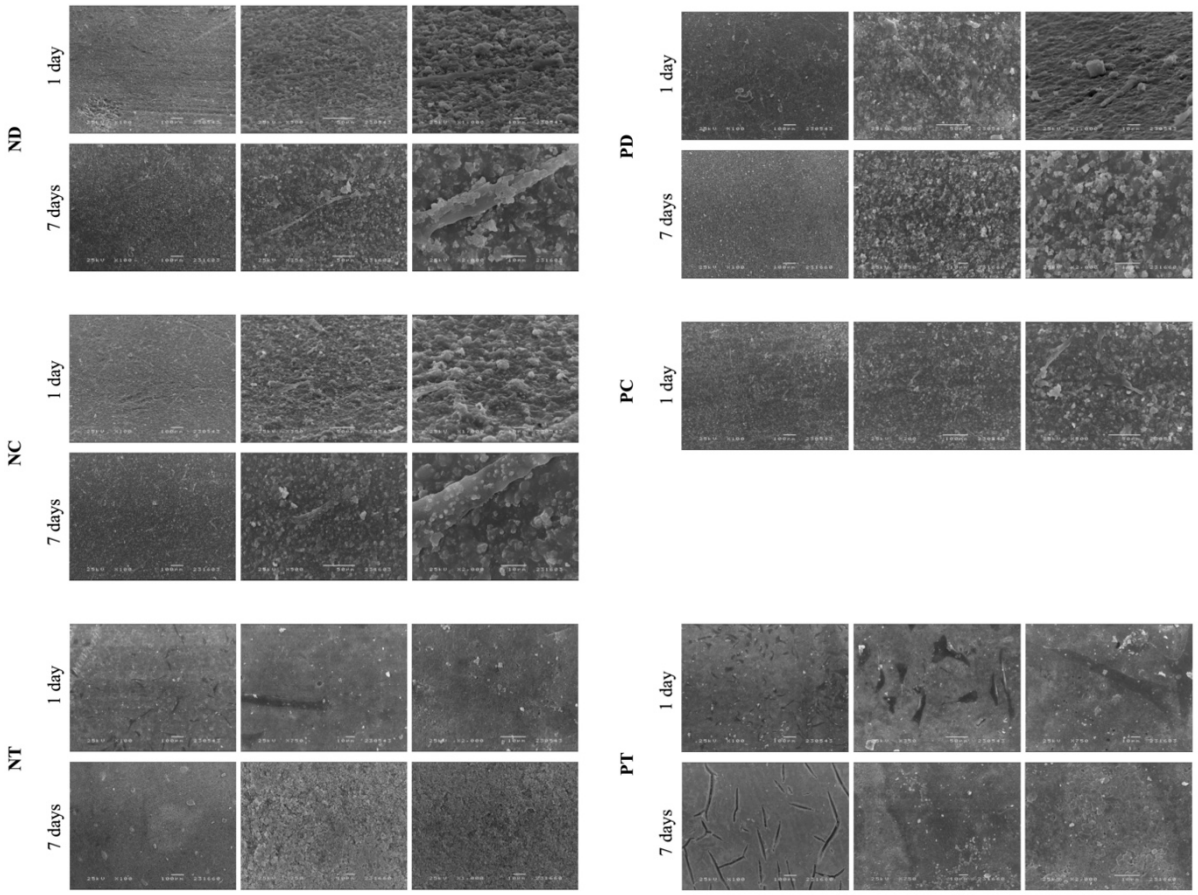

Figure 18 – FEG-SEM images of specimens cultured with HGF-hTERT of groups ND, NC, NT, PD, PC and PT at 1 and 7 days of culture.

Similarly, fluorescence microscopy images were obtained after 1 and 7 days of culture, and adherent cells were observed in all specimens, however without a true spreading of cell bodies as characteristic of fibroblasts. At day 7, for groups PC and PT, fibroblasts exhibit a spindle-like appearance, accompanied with a higher density of cells, as seen in figure 19.

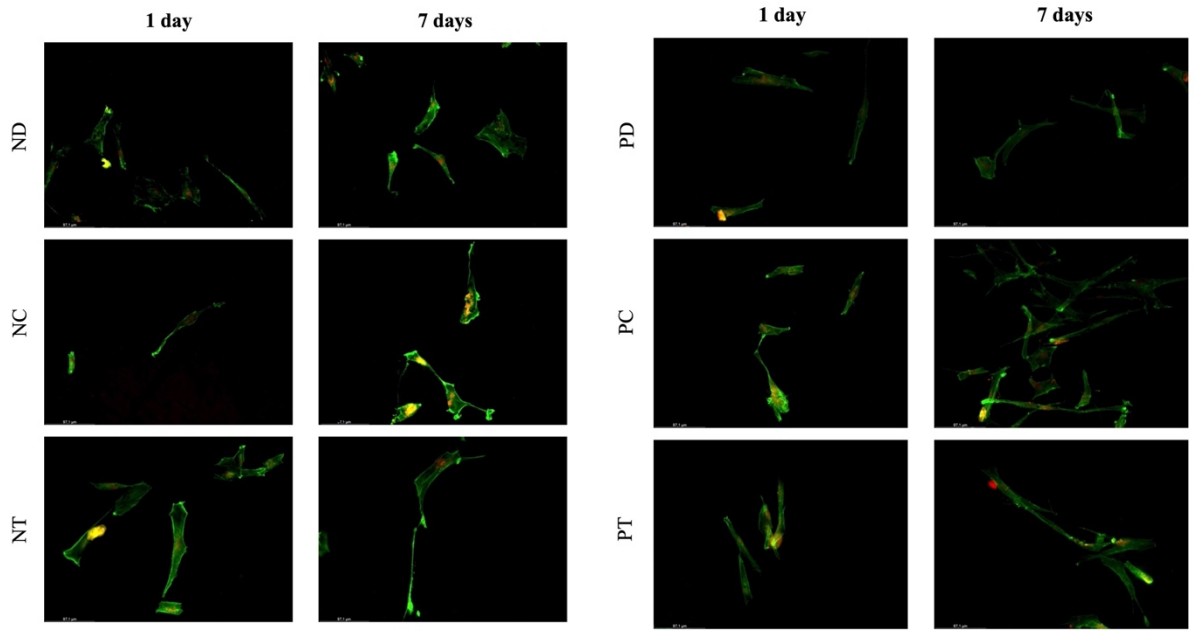

Figure 19 – Fluorescence images of specimens cultured with HGF-hTERT of groups ND, NC, NT, PD, PC and PT at 1 day and 7 days of culture (at 400x magnification, scale seen in image).

4. Contact profilometry

According to the statistical analysis, no significant differences were found between the different groups (p=0.061), as seen in figure 20. The R_a mean and SD values in μm are listed in table 2.

Table 2 - Surface roughness values for each group, presented with the mean Ra ± SD in micrometers (μm) and p value between group comparisons for Groups ND, NC, NT, PD, PC and PT.

Group	***Mean R_a (μm)***	***Standard deviation (SD) (μm)***	***p* value (group comparisons)**
ND	0.723167	0.1663771	ND-NC = 1.000 ND-NT = 0.262 ND-PD = 0.997 ND-PC = 0.984 ND-PT = 0.367
NC	0.710750	0.1825256	NC-ND = 1.000 NC-NT = 0.241 NC-PD = 0.988

			NC-PC = 0.961 NC-PT = 0.342
NT	0.432000	0.0772528	NT-ND = 0.262 NT-NC = 0.241 NT-PD = 0.094 NT-PC = 0.088 NT-PT = 0.998
PD	0.779125	0.1555883	PD-ND = 0.997 PD-NC = 0.988 PD-NT = 0.094 PD-PC = 1.000 PD-PT = 0.131
PC	0.807833	0.1497250	PC-ND = 0.984 PC-NC = 0.961 PC-NT = 0.088 PC-PD = 1.000 PC-NT = 0.123
PT	0.479375	0.1663778	PT-ND = 0.367 PT-NC = 0.342 PT-NT = 0.998 PT-PD = 0.131 PT-PC = 0.123

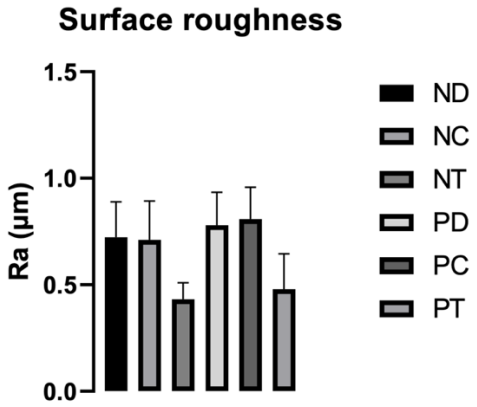

Figure 20 - Bar chart depicting surface roughness results as mean ± SD in micrometres (µm) for Groups ND, NC, NT, PD, PC and PT. Error bars represent the SD and a one-way ANOVA with Tukey's post-hoc test was used for comparison between groups.

DISCUSSION

AM is considered a comparable alternative to SM, but there is a lack of studies about cellular behaviour and biocompatibility of different 3D printers and base materials. Therefore, this study was designed to evaluate the behaviour of fibroblasts in 3D printed resin surfaces, using two different printers and three different resins.

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 between 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 M *et al*.[16]. 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 favourable physical conditions for cell attachment and proliferation as compared to control. However, similar conditions between 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 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 was only a significant difference between the Denture 3D+® resin and the Crowntec® resin and 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 Crowntec® being suitable for permanent crowns as well), rather than denture base materials, which are in a more 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. 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. Both the PD group and ND group used the Denture3D+® resin.

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 for permanent crowns (as is the case of Crowntec® resin). This was not expected, given the fact that it 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[34,35]. Guerrero-Gironés J *et al*. 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[36]. Frasheri I *et al.* concluded that 3D-printed materials, which included the NextDent C&B MFH® resin, affected cell proliferation and induced more unfavorable effects on gingival keratinocytes[37].

When comparing the composition of the resins used in this study, all are considered class IIa biocompatibility materials, which are materials for short-term use (between 60 minutes to 30 days. However, if only used in oral, nasal or ear cavities, can be for long-term, as long as is not able to be absorved by the mucous membrane, such as restorative materials, which is in conformity with what was expected[38,39]. All of the resins had a different composition, with all ingredients of Denture3D+® being in the C&B MFH® resin, although 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, although 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 each ingredient 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 (see table S1 in supplementary materials) .

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 specimens, this additional polymerization step was performed, as per 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[9,10,16,17]

In the present study no polishing was performed in the specimens, since the intaglio surface of the denture is not 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.*[40]. A study by Bieger V *et al.* found that the printed specimens which 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. Based on this, the authors suggest that the printed materials should eventually only be used short-term[28]. Therefore, given our results and the previous literature, we can speculate whether resins such as the NextDent 3D+ should be considered for long-term use or if they should be kept for short-term use only.

Gingival fibroblasts play an important role in tissue homeostasis by producing and modulation immune responses through cytokine secretion[41]. Cytokines have pro-inflmmatory functions, such as IL-6 and IL-8, or anti-inflamatory 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 major mediator of the inflammatory response and acts as a chemoattractant, inducing a neutrophil migration[33,42,43].

When comparing the concentration of inflammatory mediators, such as IL-6 and IL-8, there is a significant difference between 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 3D+® resins have a 1-5% w/w of diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO), which is the photoinitiator 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 photoinitiators, such as camphorquinone. This may explain why NT and PT groups had the lowest mean values of IL-6 and IL-8. Additionally, since photoinitiators initiate polymerization, if part remains unreacted, a lower degree of conversion is expected, which leads to a higher concentration residual monomer[43–45]. For this reason, it may indicate that the NextDent® 5100 printer could eventually lead to a lower degree of conversion. Finally, in this study a similar cellular proliferation was reported in contact with C&B MFH® resin and Crowntec® resin, whilst the latter being a more cost-effective option.

Since surface roughness is a significant variable affecting cellular behavior, it was evaluated in a representative set of specimens from each group. No significant differences were found between the groups, leading to the conclusion that the recommended printer by the mnufacturer and the third-party printer led to similar roughness properties. There was a difference in surface roughness in both groups which used the Crowntec® resin, however it failed to be statistically significant. A 2021 by Srinivasan M *et al.* also evaluated the surface roughness of 3D-printed specimens, using the NextDent Denture 3D+® resin and printed 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[16]. Given that our study compared two different printers than the ones used in the study by Srinivasan M *et al.*, no comparison can be established, and we can only conclude that there were no significant difference in the printers used in our study.

To our knowledge this is the first time that the biological effects of 3D printed dental resins produced using consumer versus high-end 3D Printers were studied. Three different resins for distinct purposes in dental medical device production were used and printed in a consumer and in a high-end 3D Printer 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 SLA 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, 2022 a study by Ziad *et al.* found no statistically significant differences between the surface roughness of specimens produced with three different 3D-printing resins, which included the NextDent Denture 3D+® resin[46].

Similarly, a comparison between 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 felt out of the scope of this study. However, a 2022 study by Atria et al. compared the mechanical characteristics between the Crowntec® and NextDent C&B MFH® resins, among others, 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[47]. Another potential concern was the inability to access the complete list of ingredients for all resins, but especially the Crowntec® resin, as to research the color pigments used, for example, and its relation to cellular biocompatibility. Finally, this was an *in vitro* study and using cells in culture which are not 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 expand the research on the *in vitro* cellular behaviour of human fibroblasts to functional parameters and use primary cell lines and othe cell types as well. Mechanical effects should also be studied along with the effect of different post-processing protocols, that may influence the amount of residual monomer present in the resin, which in turn 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.

CONCLUSIONS

Within the limitations of the present study, the following conclusions can be drawn:

1. The use of different 3D printers with equivalent parameterization does not influence the *in vitro* cellular behaviour of human fibroblasts.
2. The use of different 3D-printing resins influences moderately the *in vitro* cellular behaviour of human fibroblasts.
3. The use of different 3D printers or resins does not significantly influence surface roughness, considering the set of resins and equipments tested within this study.

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SUPPLEMENTARY MATERIALS

Table S1 - 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	<0,1	Acute Tox. 4 (Oral), H302 Eye Irrit. 2, H319

	Hydroquinone monomethyl ether		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