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In vitro cytotoxicity assessment of the 3D printed polymer based epoxy resin intended for use in dentistry

In vitro procena citotoksičnosti 3D štampanog polimera na bazi epoksi smole namenjenog za upotrebu u stomatologiji

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Abstract

Background/Aim. There is limited published evidence on the cytotoxicity of 3D printed polymer materials for dentistry applications, despite that they are now widely used in medicine. Stereolithography (SLA) is one of the foremost 3D processes used in 3D printing, yet there are only a small number of resin materials reported to be suitable for medical applications. The aim of this study was to investigate, *in vitro*, the cytotoxic effect of the 3D printed resin in order to establish the suitability for its usage in dentistry and related medical applications such as surgical dental guides, occlusal splits and orthodontic devices. **Methods**. To examine the cytotoxicity of the 3D printed polymer-based epoxy resin, Accura® ClearVue™ (3D-Systems, USA), two cell cultures were used: mouse fibroblasts L929, and human lung fibroblasts MRC-5. The cell viability was determined by the Mosmann's colorimetric (MTT) test and the agar diffusion test (ADT). **Results.** Direct contact of the tested material with the ADT test showed nontoxic effects of tested material in any cell culture. The tested material showed no cytotoxic effect after 3 days of extraction of the eluate by the MTT, but mild cytotoxic effect after 5, 7 and 21 days on both cell lines. The cytotoxicity increased with increasing the time of the eluate extraction. **Conclusion.** The 3D printed polymer-based epoxy resin, Accura® ClearVue™ (3D-Systems, USA) is considered appropriate for making surgical dental implant guides according to the cytotoxic behavior. According to the mild level of cytotoxicity after the longer extraction periods, there is a need for further evaluation of biocompatibility for its application for the occlusal splints and orthodontic devices.

Key words:
cell culture techniques; dental materials; epoxy resin; material testing; printing, three-dimensional.

Apstrakt

Uvod/Cilj. Malo je objavljenih dokaza o citotoksičnosti 3D štampanih polimernih materijala za upotrebu u stomatologiji, bez obzira na njihovu sve širu primenu u medicini. Stereolitografija (SLA) jedan je od najvažnijih 3D procesa koji se primenjuje za 3D štampu, ali postoji samo mali broj materijala na bazi smola za koje je dokazano da su pogodni za medicinsku primenu. Cilj ove studije je bio da se ispita, *in vitro*, citotoksični efekat 3D štampanog polimera kako bi se utvrdila mogućnost za njegovu upotrebu u stomatologiji i srodnim medicinskim oblastima, kao što su hirurške dentalne vođice, okluzalni splintovi i ortodontski aparati. **Metode.** Da bi se ispitala citotoksičnost 3D štampanog polimera na bazi epoksi smole, Accura® ClearVue ™ (3D-Sistems, USA), korišćene su dve ćelijske kulture: fibroblasti miša L929 i humani fibroplasti pluća MRC-5. Vijabilnost ćelija utvrđena je Mosmannovim kolorimetrijskim testom (MTT) i testom difuzije agara (ADT). **Rezultati.** Direktan kontakt testiranog materijala ispitan pomoću ADT pokazao je da materijal nije imao citotoksičan efekat ni na jednu ćelijsku kulturu. Testitrani materijal je imao blag citotoksični efekat posle 5, 7 i 21 dana ekstrakcije eluata primenom MTT na obe ćelijske linije. Citotoksičnost je rasla sa produženjem vremena ekstrakcije eluata. **Zaključak.** 3D štampani polimer na bazi epoksi smole, Accura® ClearVue ™ (3D-Sistems, USA) se može smatrati pogodnim za izradu hirurških dentalnih implantnih vođica sa tačke

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gledišta njegovog citotoksičnog uticaja. Zbog pokazanih blagih citotoksičnih efekata nakon dužih ekstrakcionih perioda eluata potrebna su dalja istraživanja u oblasti biokompatibilnosti materijala da bi se taj polimer mogao koristiti za izradu okluzalnih splintova i ortodontskih aparata.

Ključne reči:
ćelije, kultura; stomatološki materijali; smole, sintetičke; materijali, testiranje; štampanje, trodimenzionalno.

Introduction

Manufacturing processes that create physical three-dimensional (3D) objects by successive deposition or adding layers of material are commonly known as the Additive Manufacturing (AM) or 3D printing. There is a wide range of 3D printing processes capable of fabricating in polymer and metal materials, some of which are used in dental applications [1]. The various types of additive manufacturing include the stereolithography (SLA), fused deposition modeling (FDM), selective laser sintering (SLS), laminated object manufacturing (LOM), polymer jetting (polyjet) technology, Electron Beam Melting (EBM) and laser melting (LM) [2–5]. All 3D printing processes start with the 3D Computer Aided Design (CAD) geometry as an input. The CAD data is typically translated into the STL file format. Then it is sliced into a discrete number of layers, which are rebuilt using the 3D printing process. This enables the construction of parts with complex geometries and features, such as undercuts, cavities, complex internal geometry, etc., without the necessity for using molds or tools [5]. The 3D printing is widely used in medicine and related fields including: orthopedic surgery, maxillofacial and dental reconstructions, bioprinting tissues, and organs, and also for educational tools [4, 6]. Accuracy in planning and performing surgical procedures in dentistry is extremely important to achieve the desired clinical results and avoid iatrogenic damage of the surrounding tissue. Patient-specific, transient use surgical guides produced using the 3D printing allow a precise, safe and minimally invasive surgical procedure. The guides produced on the basis of 3D data obtained using cone beam computed tomography (CBCT) are specifically indicated for high-risk patients with severe orofacial anatomy such as expanded maxillary sinus or alveolar bone loss [7–10]. Data obtained from the CBCT images are transformed by the software into a format compatible with the 3D printers [8]. The surgical guides are used to place implants into the correct position and at the planned angulations [8, 9]. The 3D computer systems allow a detailed preoperative diagnosis and setting up a detailed treatment plan as well as the creation of surgical guides [10].

In recent years, implantology has become a very important field of dentistry, since patients' demand for such restorative procedures continuously grows. Consequently, the need for the surgical guides that transfer planned implant position directly into operative field is also increasing [11]. Furthermore, the development of computerized procedures led to dramatic changes and intervention options in implantology [12]. Comparing to a standard surgical procedure, a guided surgery offers high accuracy, preservation of anatomical structures as well as examination of vital structures, followed by the reduced surgical time, less invasiveness in the operation procedure and predictable prosthetic outcomes [13–15]. It was also proven that the surgical guides, produced on bases of the CBCT imaging, lead to a more accurate implant placement, comparing to conventionally fabricated guides [16]. The 3D printers appear as a logical fabrication technology for the surgical guides, since they can produce required models quickly and efficiently, revolutionizing dental and medical practice in general.

Due to the increasing requirements for treating patients with dental implants there is a need for the development of new materials for the fabrication of surgical guides by the 3D printing. Since guides come into direct contact with the open surgical wound and must stay in place during the surgical procedure, the material must be biocompatible and remain dimensionally stable during the sterilization process. To ensure a successful clinical use of materials in dentistry, it is necessary to have information about their physical, chemical, mechanical and biological properties. These properties depend not only on the chemical structure of the material and production technology but also on the behavior of materials in changeable conditions prevailing in the oral cavity [17, 18]. Dental materials and implants have special requirements such as nontoxic, non-allergic and noncancerous effects, all of which involve chemically and physically stable material [17, 19].

In vitro tests are the most appropriate strategies for determining the biological response to material and are used as the initial tests when examining biocompatibility [20, 21]. *In vitro* tests are faster, easier, cheaper, lighter and easier to repeat in comparison to other types of tests [19]. It is important that *in vitro* tests simulate the conditions *in vivo* and the conditions prevailing in the oral cavity in order to obtain significant results that can be properly interpreted [22]. In the oral environment, human gingiva cells come into contact with the components of the material directly (e.g., oral mucosa and the denture base, oral mucosa and the periosteum and surgical guides) and indirectly (oral mucosa comes into contact with the substances released in saliva). To imitate *in vivo* conditions in the presented research, the cytotoxic effects of the tested material was investigated both by the direct contact between the samples and the cells [agar diffusion test (ADT)] and by the contact of the cells with eluates of the tested material 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor (MTT test).

This research investigates used *in vitro* methods to evaluate the biological response of the SLA material Accura® ClearVue™. This material is reported by the manufacturer to be capable of meeting the United States Pharmacopeia (UPS) Class VI. The USP Class VI compounds must be made from ingredients with clear histories of biocompatibility that meet

tight requirements for leachates. The SLA process also creates 100% dense, highly cross-linked, humidity-resistant polymer parts in the Accura® ClearVue™ clear material, making it one of the most suitable for implant dentistry and related surgical applications from the aspect of their physical properties [23]. Available information on this material that originates from the manufacturer indicates its good perquisite biocompatibility and mechanical properties, but there is little scientific data on its possible citotoxicity and behavior in oral cavity.

The aim of this research was to investigate *in vitro* cytotoxic effects of the 3D printed polymer based epoxy resin Accura® ClearVue™ (3D-Systems, USA) on two different cell lines, murine L929, and human MRC-5 cell line with ADT and the MTT test, to test the increase or decrease of cytotoxicity in time and to give the answer whether the tested material can be used as material for fabrication of the 3D printed surgical dental guides, occlusal splints or orthodontic devices from the aspect of cytotoxicity.

Methods

Test material

The chemical composition of the Accura® ClearVue ™ in unpolymerised state is given in Table 1 [23].

The CAD (Magics v19, Materialise, Belgium) was used to create disk with a diameter of 5.5 mm and thickness of 3 mm (Figure 1). Thirty-six samples were analyzed. The samples were supported using the standard preparation software (Magics v19) and fabricated by the SLA-6000HD machine (3D-Systems, USA) using a 0.1 mm layer thickness. Once complete, the samples were removed from the build platform, soaked in isopropyl alcohol for 10 minutes, moved to a second isopropyl alcohol bath for 30 min, air dried before being subjected to ultraviolet light in a post-curing apparatus for 30 min to ensure full polymerization of the material. All samples were handled with gloves and were sealed in polythene back for delivery. These processes were discussed previously and the effectiveness of cleaning was established [24].The samples were cleaned and disinfected by ultrasound in an alcoholic aqueous solution, for a period of 5 min. Afterwards, they were washed in distilled water and again sonicated for a 5 min in isopropyl alcohol. The eluates (extracts) were obtained by incubation of samples in 4 mL of modified Dulbecco's solution (DMEM, Sigma) without serum, in a sealed tube at a temperature of 37 ± 1°C and in a water bath (GFL, Germany) [25, 26].

The length of the extraction period was 3, 5, 7 and 21 days. After each period of extraction, the extracts were removed and the tubes were again filled with fresh solution. All extracts were filtered off before working for sterilization purposes. During the ADT, the contact between cell lines and material samples was conducted direct, while during the MTT test it was done through eluates (extracts).

Fig. 1 – Analyzed samples.

The cytotoxicity effect was determined 24 hours after incubation of cells with the solid test samples and with the eluates from the 3rd day up to 21 days.

Cell lines

For *in vitro* biocompatibility testing, two different fibroblast cell lines (L929 and MRC-5) were used. The L929 (ATCCCRL-636) cell line was of mouse origin, whereas the MRC-5 line (ATCC CCL-171) was a human fibroblast line. The cells were cultivated in DMEM medium (Sigma, Munich, Germany) supplemented with 10% fetal calf serum (FCS) (Sigma, Munich, Germany), 2 mM L-glutamine (Sigma, Munich, Germany) and antibiotics antimycotic solution (Sigma, Munich, Germany). The cells were subcultured twice a week and a single cell suspension was obtained using 0.1% trypsin in EDTA (Serva, Heidelberg, Germany).

Mosmann's colorimetric test with tetrazolium salt

The test is based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to the products of formazan and it was used for the estimation of cell attachment and proliferation [27]. The cells were collected in the logarithmic growth phase, tripsinized, resuspended and counted in 0.1% trypan blue. The viable cells were seeded in Petri dishes (50 mm Center well, Falcon) with the tested substance/discs, in concentration of 2×10^5/mL. The control samples did not contain tested substance.

Table 1

Chemical composition of Accura® ClearVue ™ [23]

Component	%
4.4' isopropyliden 2-cycloheksanol, the oligomeric products of reaction with 1-chloro-2.3-epoxypropane	40–65
mixture of triarylsulfonium salt (50% of propylene carbonate and 50% of a triarylsulfonium hexafluoroantimonate salt)	1–10
3-ethyl-3-hydroxymethyl-oxetane	10–20

The Petri dishes with the seeded cells were left in thermostat at 37°C and 5% CO_2 for 48 h. After incubation, the cells were seeded in fresh medium. The viable cells were seeded (5×10^3/100 µL) in quadruplicates in a micro titer plate with 96 holes. The plates with the seeded cells were left at the thermostat at 37°C, 5% CO_2 for 24, 48 and 72 h [27]. Immediately prepared MTT solution was added into all holes in the plate in a volume of 10 µL per hatch and incubation was continued for the next 3 h (in the incubator at 37°C, 5% CO_2). At the end of the 3rd h, 10 µL 0.04 mol/L HCl in isopropanol was added in every hole. The absorbance was read immediately after the incubation on the micro titer plate reader (Multiscan, MCC/340) at a wave length of 540 nm and referent wave length of 690 nm. The holes on the panel that contained only medium and MTT, but no cells, were used as blanks ("blank").

Cytotoxicity was expressed in percentage according to following equation: CI(%) = 1−Ns/Nk × 100, where Nk is the number of cells in the samples and the Ns is the number of cells with tested substance.

The results are presented as percentages of viable cells (% cell viability = [total viable cells / total cells (viable + dead)] × 100). Quantitative changes in cell viability were presented descriptively: cell viability > 90% control – material is noncytotoxic; cell viability = 60–90% control – material is mildly cytotoxic; cell viability = 30–59% control – material is moderately cytotoxic; cell viability of < 30% control – material is serious cytotoxic [25].

Agar diffusion test

The agar diffusion test is carried out according to the international standard ISO 10993 and ISO 7405 [20, 21].

Eagle's Basal Medium which contained 2.2 g/L sodium bicarbonate, 3.0 g/L HEPES and 50 ml/L of serum was used in this test. Double concentration of medium was prepared without HEPES, and Na_2CO_3 1 g/L was reduced. Agar medium was sterilized by autoclaving and filtration. Neural-red solution was kept protected from light. The Petri dishes were used in diameter 100 mm suitable for the cell culture. The cells were harvested in the logarithmic growth phase.

The samples of test material in the form of discs were placed on the top layer of agar in each dish and were incubated under controlled conditions (37° C, 5% CO_2) for 24 h. After hardening of the agar nutritive medium at room temperature (about 30 min), the cells were stained with neutral red (10 mL) and left in the dark for 15 min. Staining of the cells was achieved by adding neutral red (10 mL, a solution of 0.01% neutral red in phosphate buffer) that diffuses through the agar and makes living cells intensively red. Excess of a neutral red solution was aspirated [28, 29].

The decolorization zone around the test materials and controls was assessed using an inverted microscope with a calibrated screen, and the Decolorization Index and Lysis Index were determined for each specimen in accordance with the criteria presented in Table 2.

Table 2

Decolorization index and lysis index for agar diffusion test (ADT)

Decolorization index	Decolorization	Lysis Index
0	No decolorization detectable	no cell lysis detectable
1	Decolorization only under the test substance	less than 20% cell lysis
2	Decolorization zone not greater than 5.0 mm from the test substance	20–40% cell lysis
3	Decolorization zone not greater than 10.0 mm from the test substance	40–60% cell lysis
4	Decolorization zone greater than 10.0 mm from the test substance	60–80% cell lysis
5	The total culture is decolorized	more than 80% cell lysis

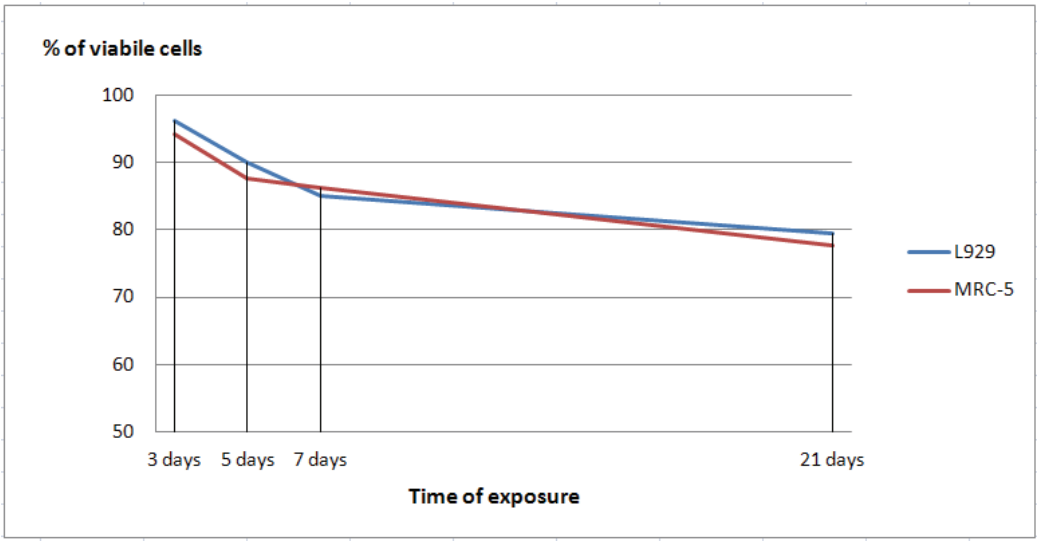

Fig. 2 – Viability of L929 and MRC-5 cell line presented as the percentage of the control found by the Mosmann's colorimetric (MTT) assay.

The results were statistically analyzed using the SPSS. The results obtained by the MTT assay and ADT are expressed as the mean (M) with a standard deviation (SD) and standard error (SE). The mean value, standard deviation and coefficient of variation (CV) were calculated for the replicates (at least triplicate) samples in each experiment. Cytotoxicity was expressed as a percentage. Comparison of the response of two cultures at different extraction period was performed using the *t* test with a significance level of $p = 0.05$.

Results

MTT test

The results of the MTT test are presented in Figure 2 and Table 2.

The results are presented as the percentage of viable cells compared to the control group without tested material.

After the incubation of murine fibroblast L929 cell line with the extracts of experimental material in DMEM, it was observed that the tested material was found noncytotoxic after the first tested period of 3 days and mildly cytotoxic after all other tested periods. After 3 days of exposition, the percentage of viable cells was 96.26%, after 5 days 89.96%, after 7 days 85.10% and after 21 days 79.40% (Figure 2 and Table 3). After the incubation of human fibroblast MRC-5 cell line with extracts of experimental material in DMEM, it was observed that the tested material was also found noncytotoxic after the first tested period of 3 days and mildly cytotoxic after all other tested periods. After 3 days of exposition, the percentage of viable cells was 94.10%, after 5 days 87.61%, after 7 days 86.21% and after 21 days 77.60% (Figure 2 and Table 3). The cytotoxic effect depends on the period of extraction and tends to rise with the period length. The highest cytotoxicity was expressed by the eluate from the 21 days, and a minimal from the 3rd day. The tests on both cell lines with the eluate of the first extraction period of 3 days showed that the material had noncytotoxic effect, as opposed to cells that were in contact with the eluate of the 5th, the 7th and the 21st day of the extraction, which showed mild cytotoxicity of tested material (Figure 2 and Table 3).

By comparing the responses of two different cell cultures, it was found that the viability of L929 murine fibroblasts was slightly higher compared to the viability of the MRC-5 human fibroblasts, for all periods of extraction except for the extraction of eluent from the 7th day of extraction, when the viability of human fibroblasts was slightly higher (Figure 2).

Agar diffusion test

The results of the agar diffusion test were obtained according to the 4 replicate tests. Cytotoxic effect of 3D printed samples from Accura® ClearVue ™ was not recorded neither on L929 cell line nor on MRC-5 cell line. There was no discoloration around the specimen in any cell culture and the lysis index was 0 (Table 3).

Table 3

The results of the Mosmann's colorimetric (MTT) assay on the L929 and MRC-5 cell line

Cell line		Days			
	C	3	5	7	21
L929	1.008	0.855	0.689	0.824	0.742
	0.962	0.915	0.457	0.769	0.781
	0.951	0.824	0.650	0.725	0.743
	0.985	0.822	0.695	0.773	0.784
	0.968	0.971	0.839	0.745	0.738
	0.860	0.895	0.760	0.798	0.764
	0.943	0.856	0.920	0.812	0.719
	0.963	0.872	0.840	0.821	0.743
Blank: 0.012					
mean ± SD	0.934 ± 0.050	0.899 ± 0.051	0.840 ± 0.065	0.794 ± 0.034	0.741 ± 0.018
Cv	5.375	5.667	7.779	4.283	2.490
Ci %	0.000	3.749	10.043	14.944	20.624
C %		96.251	89.957	85.056	79.376
MRC-5	0.539	0.525	0.475	0.572	0.442
	0.575	0.555	0.496	0.611	0.436
	0.458	0.522	0.407	0.588	0.458
	0.457	0.519	0.499	0.602	0.441
	0.599	0.477	0.409	0.540	0.412
	0.616	0.566	0.411	0.464	0.410
	0.543	0.556	0.548	0.419	0.423
	0.461	0.489	0.576	0.490	0.422
Blank: 0.034					
mean ± SD	0.555 ± 0.070	0.522 ± 0.045	0.486 ± 0.089	0.478 ± 0.051	0.431 ± 0.007
Cv	12.591	8.713	18.210	10.569	1.557
Ci %	0.000	5.904	12.393	13.790	22.397
C %		94.096	87.607	86.210	77.603

***C – control; Ci –cytotoxicity.**
SD – standard deviation; CV – coefficient of variation.

To grade the test material, the scale that takes into account the decolorization index and cell lysis index was used. The cell response was calculated by Decolorization Index divided by Lysis Index. Cell response was 0, so obtained results are considered noncytotoxic.

Discussion

The present research was made by the MTT assay in which the material was in contact with the cells by means of the eluate and the agar diffusion test in which the samples were in direct contact with the cells, as the monomers and other components diffuse through a thin layer of agar. Cytotoxicity tests are considered to be primary, *in vitro* tests, for the research of biocompatibility of materials that are to be used for medical purposes. They provide information about the cytotoxicity of the material and cytotoxicity of eventual leaking agents from the material on the cell culture [30]. They are standardized and the results can be easily compared with required levels of safety [21, 22]. According to ISO 7405 standard, both the direct contact test and the contact of the cells with eluates, are recommended [21]. With the direct contact tests, *in vivo* conditions are better imitated, but they involve a certain risk of mechanical damage of the cells and also increase the risk of bacterial contamination of the cell culture [22].

Agar diffusion method, which is less sensitive compared to the MTT test, showed a completely negative result. The MTT test showed a drop-in cell number with an increase of the length of extraction in relation to the control group. The difference in results is caused mainly due to different sensitivity of the two tests.

By comparing the cell viability of two different cell cultures in the present research, it can be noted that the higher rate of survival showed the mission in relation to human fibroblasts. The exception is the 7th day, where the MRC-5 culture cells were more viable. These differences may be related to the differences in cell lines (human and murine fibroblasts) as well as to the differences in their doubling period, but the final ranking of the results of cytotoxicity did not differ for the L929 and MRC-5 cell line. Murine fibroblasts belonging to the permanent cell cultures (L929) as well as human fibroblasts (MRC-5) can be regarded as analogues of cells of oral mucosa [31]. Murine fibroblasts are often used in various studies of dental materials [32]. Permanent cell lines are simpler systems without specific metabolic potential as target cells (target cells *in vivo*). However, some research suggests that these cells are more sensitive to a variety of tests in relation to the primary culture [32]. Fibroblast cell line MRC-5 in the present research was used to obtain the results of the human cell line and check whether there is a difference between the mouse and human fibroblasts in terms of cellular response. Thus, the obtained results may be interpreted in a clinically relevant manner.

The MTT test carried out on the cell culture MRC-5 showed that the material had a satisfactory degree of biocompatibility, which was not less than 77.60% and more than 94.10% cell viability. The lowest recorded viability was 77.60% and this value represents the viability of MRC-5 culture in contact with eluates from the 21st day. The fibroblasts viability of both cell lines measured by the MTT assay was the lowest in the most concentrated eluate.

The tested material showed no cytotoxic effect in direct contact with the cells and after 3 days of the extraction period. This finding indicates that the 3D printed polymer-based epoxy resin, Accura® ClearVue™ (3D-Systems, USA) can be used for the production of surgical dental guides that are to be in the contact with the wound or mucosa for a short period of time (up to a couple of hours). Findings of the mild cytotoxic effect after 5, 7 and 21 days of extraction and increasing cytotoxic effect with increasing the time of extraction indicate that the tested material could be used even for production of occlusal splints and orthodontic devices, but the additional tests of biocompatibility are necessary, because of the long term contact of those dental devices with oral mucosa.

Similar tests conducted on the acrylic materials, denture base materials used for decades, showed mild to moderate cytotoxicity [28, 29]. The release of toxic substances from the acrylate is most intense in the first 24 hours after polymerization. Research show that greatest cytotoxicity of composite resin can be expected in the first 24 hours after polymerization, in contrast to the presented study where the cytotoxicity was the lowest in the first 3 days, but increased with the period of extraction.

In addition to the chemical composition of materials, the method of polymerization is one of the decisive factors as far as the cytotoxicity of acrylic resin is concerned [33]. An extension of the polymerization time can reduce the toxicity of the resin. The Accura® ClearVue ™ material is continuously polymerized during the SLA process, then further post-cured after being cleaned [3]. It can be assumed that this method of polymerization contributes to reduction of the amount of residual monomer and consequently better biocompatibility of epoxy resin.

Although the epoxy-based material evaluated in this research met prerequisite material and processing standards for medical applications, similar cross-linking acrylic materials were proven to be potentially toxic, mutagenic or allergenic [34]. Some studies showed that the 3D printed objects can be toxic [35, 36], allergenic [15, 36] , or cause inflammatory response combined with infections [31]. Besides adverse reaction to the generated product, some hazardous particles can be found in the air during specimen production [31, 37]. It is, therefore notable that very few research articles discussing the biocompatibility issue of the 3D printed materials can be found in the available literature [37, 38]. A recent study, where toxicity of the 3D printed parts was assessed on zebra fish embryos, concluded that both FDM and SLA samples were measurably toxic [37]. Furthermore, the SLA specimens showed greater toxic potential, that could, however, be greatly reduced by post-processing of samples by means of the UV light exposure. Though direct comparison is not implementable since different resin was used opposed to present research, safety data sheets indicate that it is likely the both materials contain the acrylic and/or metacrylic groups. Another study [38] on the same culture also revealed a potential health risk that can be reduced by a post-printing treatment of the specimens. Ho-

wever, it has to be noted that human application differs from aquatic animals; therefore implication of further tests is necessary.

With a growing field of 3D printers, their size reduction to a desktop variant would certainly make the surgical guides more reachable for everyday clinical use. Those guides are in a limited contact with live tissues (< 24 h), yet clinical situation might demand bone or blood communication, which might require sensitization, irritation or systemic toxicity tests, according to ISO 10993. Despite many advantages of its application, biocompatibility of printed parts still remains a field for further investigation.

Conclusion

Within the limitations of this study it was concluded that the 3D printed Accura® ClearVue™ material had non-cytotoxic effect on the cell cultures L929 and MRC-5 in direct contact with the cell culture tested by the ADT. MTT assay showed no cytotoxic effect after 3 days of extraction of eluates and mild cytotoxic effect after 5, 7 and 21 days of extraction on both cell cultures. Cytotoxicity increases with the longer extraction patterns. Human cell line was somewhat more sensitive to the action of the material, but it is not clinically relevant. The 3D printed polymer-based epoxy resin, Accura® ClearVue™ (3D-Systems, USA) is considered appropriate for making surgical dental implant guides according to the cytotoxic behavior. According to the mild level of cytotoxicity after the longer extraction periods, there is a need for further evaluation of biocompatibility for its application for occlusal splints and orthodontic devices.

Further research shall be focused on *in vivo* testing to reach comprehensive data about biocompatibility and potential use of the 3D printed epoxy resin in different fields of dentistry.

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