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SCIENTIFIC ARTICLE

PROMENE NA HELA ĆELIJSKOJ KULTURI U PRISUSTVU AKRILATA ZA BAZU PROTEZE

CHANGES ON HELA CELL CULTURE IN PRESENCE ON ACRILIC RESINS

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Kratak sadržaj

Akrilati su često upotrebljavani materijali u stomatoprotetskoj praksi, posebno u izradi baza zubnih proteza. U pojedinim slučajevima uočava se reakcija oralnih tkiva na mestima kontakta sa bazom akrilatnih proteza.
Materijal za ispitivanje je obuhvatao četiri različita akrilata za bazu proteze. Ispitivan je uticaj ekstrakata akrilata različitih koncentracija na vijabilnost HeLa ćelija, kao i reverzibilnost nastalih promena na ćelijskoj kulturi. Kao kontrola je služila kultura koja je rasla u medijumu bez ekstrakata. Procena vijabilnosti HeLa ćelija vršena je MTT testom.
Sa porastom koncentracije ekstrakata ispitivanih akrilata vijabilnost HeLa ćelija značajno opada, a i usporeniji je njihov oporavak. Potpuni oporavak HeLa ćelija nije primećen ni u jednoj od ispitivanih koncentracija.

Ključne reči: *akrilati, baza proteze, HeLa ćelijska kultura, MTT test*

Abstract

Acrylic resins are materials often used in dental practice, especially in denture base manufacturing. In some cases, a reaction of the oral tissues appears on the contact spots with the base of acrylic dentures.
Testing materials considered of four different acrylic resins. The influence of differently concentrated acrylic extracts on the viability of HeLa cells was examined, together with the reversibility of the changes which appeared on cells' culture. A culture that grew in an extract free medium was used as control. The estimation of HeLa cells' viability was done by the MTT test.
As the concentration of examined acrylic extracts grows, the viability of HeLa cells considerably declines, and their recovery is slower. Complete recovery of HeLa cells has not occurred in any concentration of all examined.

Key words: *acrylic resins, denture base, HeLa cell culture, MTT test*

Uvod

Poli(metil metakrilat) (PMMA) predstavlja danas najčešće korišćeni materijal za izradu baze proteze, opturator i maksilofacijalnih proteza, ortodontskih aparata, kao i za potrebe njihovih podlaganja i reparatura[1,2,3]. Razlozi za njihovu značajnu primenu jesu korektne fizičke i mehaničke osobine, transparentnost kao i relativno laka manipulacija [4]. U svakodnevnoj praksi, najčešće se upotrebljavaju toplo i hladnopolimerizujući akrilati koji se, s obzirom na ulogu morfološkog i funkcionalnog supstituenta u usnoj duplji, svrstavaju u grupu biomaterijala[5].

Introduction

Poly (methyl methacrilate) (PMMA) is, at present, the most frequently used material in manufacturing denture bases, obturator and maxillofacial dentures, orthodontic devices, and also, for their relining and reparation[1,2,3]. There are numerous reasons for its applicability, such as its physical and mechanical features, transparency and the possibility of relatively easy manipulation[4]. Heat-cured and cold-cured acrylic resins are most commonly used in every day practice. As they play the role of a morphological and functional substitute in oral cavity, they are placed into the group of biomaterials[5].

Potencijalno toksične supstance iz akrilata vremenom se oslobađaju iz površnih slojeva protezne baze i difunduju u pljuvačku i, ne retko, izazivaju inflamatorne a ređe i alergijske reakcije mekih tkiva sa kojima dolaze u kontakt [6,7].

Patološke promene se klinički manifestuju kao kontaktni stomatit (*stomatitis protetica*), stomatodinije i sor (*candidiasis*)[8,9]. Smatra se da čak 17% korisnika pločastih zubnih proteza ispoljava preosetljivost na akrilate[1]. Veoma retko mogu nastati i sistemska oštećenja [10].

Procena tolerancije nekog materijala od strane živog tkiva može se izvršiti, između ostalog i na osnovu testiranja njihove biokompatibilnosti *in vitro* metodama. *In vitro* testovi citotoksičnosti predstavljaju preliminarne testove za procenu biokompatibilnosti materijala [2,3,11]. Najzastupljeniji biološki sistemi za testiranje toksičnih efekata stomatoloških materijala jesu ćelijske kulture. Prednost ispitivanja *in vitro*, u poređenju sa studijama na eksperimentalnim životinjama, je u mogućnosti ponavljanja pod identičnim uslovima, strogoj kontroli po svakom parametru i ekonomskoj isplatljivosti [12].

Cilj rada bio je da se ispita efekat različitih koncentracija ekstrakata akrilata na vijabilnost *HeLa* ćelijske kulture, kao i mogućnosti njenog oporavka.

Materijal i metode

Materijal za ispitivanje su činila četiri različita akrilatna materijala (tabela 1).

Napravljeno je po 5 uzoraka od svake grupe (*n*=20) istih dimenzija (15 x 10 x 2mm).

U istraživanju je korišćena *HeLa* S3 ćelijska linija (American Type Culture Collection, Rockville, MD, SAD) koja se smatra analogom epitelnih ćelija oralne mukoze.

Ekstrakti akrilata dobijeni su inkubacijom u DMEM-u (Dulbecco's Modified Eagle's Minimal Essential Medium, PAA Laboratories GmbH) u odnosu 0.2gr akrilata u 5ml medijuma (ISO 10993: 1998)[13]. Ekstrakcija uzoraka vršena je u zatvorenim plastičnim epruvetama na temperaturi od 37±1^{0}C u vodenom kupatilu, u trajanju od 3 dana. Napravljene su koncentracije ekstrakata od 10%, 25%, 50% i 100%. Efektivne koncentracije ekstrakata su bile dvostruko manje, jer su ekstrakti dodavani na istu

Potentionally toxic substances from the acrylic resins are being leached from the surface layers of the denture base and diffunded in the saliva, which often causes inflammatory reactions, and rarely, allergic reactions of soft tissues to which the toxic substances come in contact[6,7].

Pathological changes are clinically manifested as stomatitis protetica, stomatodynia and candidiasis[8,9]. It is considered that even 17% of dentures' users are sensitive to acrylic resins[1]. Systematic damages can appear, too, but very rarely[10].

Tolerance that a live tissue may create against some material can be estimated according to the testing of their biocompatibility using *in vitro* methods. *In vitro* cytotoxicity tests represent preliminary tests for estimation of materials' biocompatibility[2,3,11]. Most applied biological systems for examining the toxic effects of dental materials are cell cultures. When compared to studies on experimental animals, the *in vitro* method has a major advantage which lies in the possibility of repetition under identical conditions, precise control of every parameter and financial benefit[11].

The purpose of the study was to examine the effect of different concentrated acrylic extracts on the viability of *HeLa* cell culture, and the possibility of its recovery.

Material and methods

The testing material consisted of four different acrylic resins (table 1).

Five samples with same dimensions (15x10x2mm) were made from each group (n=20).

HeLa S3 cell line (American Type Culture Collection, Rockville, MD, USA) was used in research, a cell line considered to be analogous to epithelial cells of oral mucosa.

Acrylic extracts were obtained by incubation in DMEM (Dulbecco's Modified Eagle's Minimal Essential Medium, PAA Laboratories GmbH) in proportion of 0.2g of acrylic resin in 5ml of medium (ISO 10993:1998)[13]. The extraction of samples was performed in closed plastic vials, at 37±1^{0}C, in water bath for 3 days. 10%, 25%, 50% and 100% extracts' concentrations was made. The effective concentrations of the extracts were being added to the same

Tabela 1. *Ispitivani akrilati za bazu proteze*
Table 1. *Tested acrylic resins*

Naziv akrilata i proizvođač Resins' name and manufacture	***Način polimerizacije Polymerization type***	***Sastav Components***		***Odnos praha i tečnosti (g/ml) Powder/liquid ratio (g/ml)***
		polimer polymer	***monomer monomer***	
Simgal-R Galenika, Srbija	15-18min. na 22^0C 15-18min. at 22^0C	PMMA	MMA	dodavati prah do zasićenja Powder to saturation
Triplex Cold Ivoclar Vivadent, Lihtenštajn	15 min. na 22^0C 15 min. at 22^0C	PMMA	MMA, EGDMA	13:10
Biocryl - RN Galenika, Srbija	30 min na 70°C, 30min na 100°C 30 min at 70°C, 30min at 100°C	PMMA	MMA	20:10
Triplex Hot Ivoclar Vivadent, Lihtenštajn	45min na 100°C 45min a 100°C	PMMA	MMA, EGDMA	23,4:10

zapreminu medijuma sa ćelijama. Svi ekstrakti su sterilisani filtracijom kroz 0,2 μm filter.

Ćelije su zasađene u tri sterilne ploče za kultivaciju sa 96 mesta. U svako pojedinačno mesto sađeno je po $2x10^4$ ćelija u 50 μl DMEM-a sa dodatkom 2mM l-glutamina, 100 IU/ml penicilina, 100 GU/ml streptomicina (PAA Laboratories GmbH) i 10% fetalnog goveđeg seruma (Gibco, UK), na koje je dodato još 50 μl ekstrakata četiri akrilata za bazu zubne proteze različitih koncentracija. Kontrola je sadržala $2x10^4$ ćelija u 100 μl DMEM-a. Ispitivanje za svaki od materijala vršeno je u kvadriplikatu. Ćelije su inkubirane 3 dana, u atmosferi zasićenoj vodenom parom, sa 5% CO_2 na 37ºC. Potom je utrađen MTT test.

Da bi se ispitala reverzibilnost nastalih promena nakon navedenog vremenskog perioda, iz jedne od ploča za kultivaciju ćelija izvučen je medijum sa ekstraktima materijala iz svih pojedinačnih mesta i zamenjen svežim DMEM-om. U drugoj ploči zamena je izvršena sa po 100 μl ekstrakata četiri akrilata za bazu proteze odgovarajućih koncentracija. Sledila je trodnevna inkubacija. Nakon ukupno 6 dana inkubacije urađen je MTT test.

MTT test zasniva se na aktivnosti enzima sukcinat-dehidrogenaze koji je sastavni deo mitohondrijalnog respiratornog lanca vijabilnih ćelija. Navedeni enzim redukuje žutu tetra-

volume of medium with cells. All extracts were sterilized by filtration through a 0.2μm filter.

The cells were placed in three sterile tissue culture plates with96 wells. In each individual well, $2x10^4$ cells in 50μl of DMEM were placed, with addition of 2 mM l-glutamine, 100UI/ml penicillin, 100GU/ml streptomycin (PAA Laboratories GmbH) and 10% of fetal bovine serum (Gibco, UK), to which another 50μl of four acrylic resins' extracts were added, all of different concentrations. The control consisted of $2x10^4$ cells in 100 μl DMEM. The experiments for each material were done in a quadruplicate. The cells were incubated for 3 days in a fully humidified atmosphere with 5% CO_2 at 37^0C. MTT test followed.

To examine the reversibility of the newly formed changes after the appointed time, the medium with materials' extracts was removed from all the wells in one tissue culture plate, and replaced by a fresh DMEM. In a second plate, the replacement was done with 100 μl of the four acrylic resins' extracts each, and corresponding concentration. 3 days' incubation period followed. After total incubation time of 6 days, an MTT test was performed.

MTT test is based on the activity of succinate-dehyrogenases enzyme, which is constituent part of the mitochondrial respiratory cycle of viable cells. The enzyme mentioned reduces the yellow tetrazolium salt ((3-(4, 5-dimethyltiazolil-2)-2, 5-diphenyltetrazolijum bromide-MTT)

zolijumovu so ((3-(4, 5-dimetiltiazolil-2)-2, 5-difeniltetrazolijum bromid-MTT) do formazana, jedinjenja plave boje koje se u vidu kristala taloži u ćelijama. Medijum u kome su inkubirane ćelije izvučen je po završetku šestodnevne inkubacije, ćelije su isprane sa 100 µl PBS-a (Phosphate Buffered Saline) i dodato je po 20 µl MTT-a. Nakon 4h inkubacije na 37ºC, nastali kristali formazana rastvoreni su dodatkom 100 µl izopropanola. Spektrofotometrijsko merenje redukcije MTT-a vršeno je na optičkoj gustini od 540 nm, na višekanalnom fotometru (Multiskan Ascent Nº354, Thermo Labsystems, Finska). Spektrofotometrijski očitan intenzitet plave boje, nakon ekstrakcije formazana, direktno je proporcionalan broju vijabilnih ćelija.

Eksperiment je ponovljen dva puta.

Za statističku obradu podataka korišćena je analiza varijanse (ANOVA). Statistički značajnim smatrani su nivoi od $p<0,05$.

Rezultati su prezentirani kao procenat vijabilnosti ćelija. Vijabilnost ćelija kontrolne grupe predstavljen je kao 100%.

Kvantitativne promene u vijabilnosti ćelija nakon inkubacije u ekstraktima akrilata predstavljene su i opisno:[14]

1. netoksični akrilati: vijabilnost ćelija>90% kontrole,
2. blago citotoksični akrilati: vijabilnost ćelija 60-90% kontrole,
3. umereno citotoksični akrilati: vijabilnost ćelija 30-59% kontrole, i
4. ozbiljno citotoksični akrilati: vijabilnost ćelija <30% kontrole.

Rezultati

Nakon kultivacije *HeLa* ćelija u uzorcima akrilata dobijenih trodnevnom ekstrakcijom u DMEM-u uocena je negativna korelacija između koncentracije ekstrakata akrilata i vijabilnosti ćelija (grafikon 1). Srazmerno sa porastom koncentracije ekstrakata došlo je do pada ćelijske proliferacije, što govori u prilog njihovoj toksičnosti. Statistički značajan pad vijabilnosti ćelija prisutan je kod svih tipova akrilata ($p<0,01$), osim kod Biocryl-a RN.

Procenti vijabilnosti *HeLa* ćelija određeni MTT testom, prilikom ispitivanja reverzibilnosti nastalih promena, prikazani su na grafikonima 2-5.

Statistički značajna razlika u vijabilnosti kultura kojima su nakon tri dana ekstrakti

to formasan, a blue chemical compound witch makes a crystal-like layer inside the cells. The medium with the incubated cells was taken out after 6 days' long incubation. The cells were washed out with 100 µl of PBS (Phosphate Buffered Saline) and with 20 µl MTT added to each. After another 4 hours of incubation at 37^0C, the newly created formasan crystals were soluted with 100 µl of izopropanol. Spectrophotometrical measuring of MTT reduction was performed at an optical density of 540 nm, on a multichannel photometer (Multiskan Ascent Nº354, Thermo Labsystems, Finland). Spectrophotometrically viewed, the intensity of blue, after formasan extraction, is in direct proportion to the number of viable cells.

The experiment was performed twice.

The analysis of variance (ANOVA) was used for statistical data examining. Levels of $p<0.05$ were treated as statistically significant.

The results were presented as the percentage of cells' viability. The cell viability of the control group was presented as 100%.

Quantitative changes in cells' viability after incubation in the acrylic extracts are presented descriptively[14]:

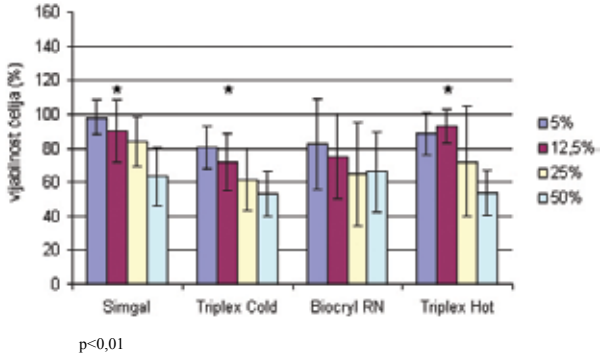
1. non-cytotoxic acrylic resins: cell viability >90% in relation to control;
2. slightly acrylic resins: cell viability 60-90% in relation to control;
3. moderately acrylic resins: cell viability 30-59% in relation to control, and
4. severely acrylic resins: cell viability <30% in relation to control.

Results

After the cultivation of *HeLa* cells in acrylic samples obtained after 3 days' extraction in DMEM, it has been observed that there existed a negative correlation between the acrylic extracts' concentration and cells' viability (graph 1). Proportionally, as the concentration of the extracts increased, the cell proliferation decreased, witch indicates their toxicity. Statistically significant decline of cell viability is present with all acrylic types ($p<0.01$), expect with Biocryl RN.

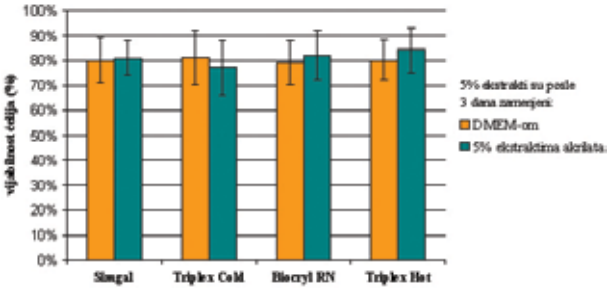
The percentage of *HeLa* cell viability, determined by MTT test while testing the newly formed changes' reversibility, is shown in graphs 2-5.

Statistically significant difference between the viability of cultures to which acrylic extracts were replaced, after 3 days, by DMEM and those, to which the extracts were replaced



Grafikon 1. *Vijabilnost ćelija nakon trodnevne inkubacije u ekstraktima akrilata*

Graph. 1. *Cell viability after 3 days' incubation in acrylic extracts*



Grafikon 2. *Vijabilnost HeLa ćelija nakon inkubacije u 5% ekstraktima akrilata*

Graph. 2. *HeLa cell viability after incubation in 5% acrylic extracts*

akrilata zamenjeni DMEM-om i onih kojima su ekstrakti zamenjeni istim, 5% ekstraktima akrilata nije uočena ni kod jednog od ispitivanih uzoraka.

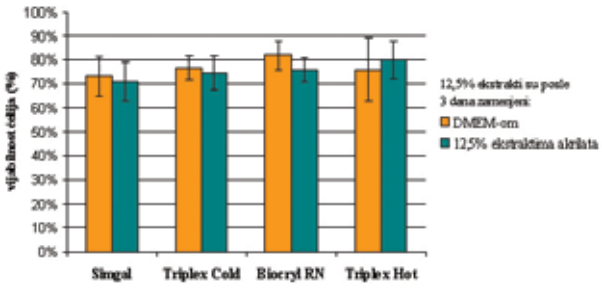
Svi ispitivani akrilati koncentracije 5% pokazali su blagi citotoksični efekat.

Vijabilnost ćelija veća je u slučaju kad su 12,5% ekstrakti akrilata zamenjeni čistim medijumom, osim u slučaju Triplex Hot-a. Ni u jednom od ispitivanih slučajeva nema statistički značajne razlike.

Svi ispitivani 12,5% ekstrakti akrilata pokazali su blagi citotoksični efekat.

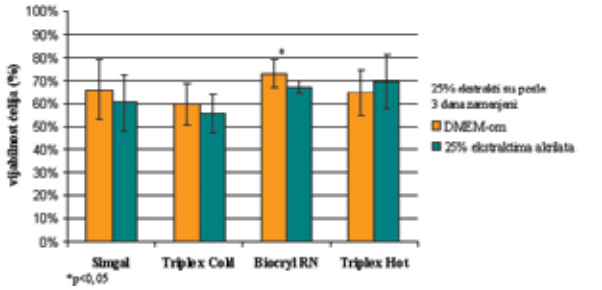
Pri koncentraciji akrilata od 25%, vijabilnost ćelija takođe je veća u slučaju zamene medijuma DMEM-om sa izuzetkom Triplex Hot-a. Statistička značajnost postoji kod uzoraka Biocryl RN-a.

Svi akrilati, osim Triplex Cold-a, pokazali su blagi citotoksični efekat pri koncentraciji od 25%. Ekstrakti Triplex Cold-a ispoljili su ume-



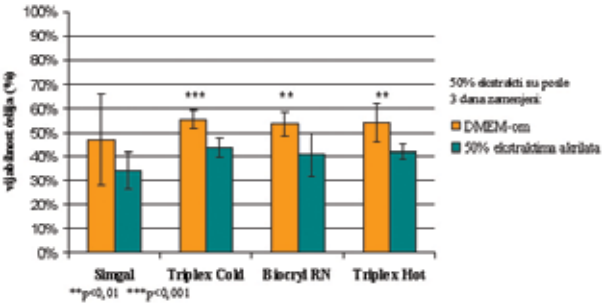
Grafikon 3. *Vijabilnost HeLa ćelija nakon inkubacije u 12, 5% ekstraktima akrilata*

Graph. 3. *HeLa cell viability after incubation in 12. 5% acrylic extracts*



Grafikon 4. *Vijabilnost HeLa ćelija nakon inkubacije u 25% ekstraktima akrilata*

Graph. 4. *HeLa cell viability after incubation in 25% acrylic extracts*



Grafikon 5. *Vijabilnost HeLa ćelija nakon inkubacije u 50% ekstraktima akrilata*

Graph. 5. *HeLa cell viability after incubation in 50% acrylic extracts*

by same 5% acrylic extracts, was not detected with any of examined samples.

All tested acrylic resins with concentration of 5% showed o slight cytotoxic effect.

Cell viability is at higher rate in cases where 12.5% acrylic extracts are replaced by pure medium, except in the case of Triplex Hot. There is not statistically significant difference in any of the situations tested.

All tested acrylic extracts of 12.5% showed a slight cytotoxic effect.

At acrylic concentration of 25%, cell viability is at a higher degree in cases when the medium is replaced by DMEM, with the exception

reni toksični efekat, što govori u prilog veće toksičnosti hladnopolimerizujućih akrilata.

Pri najvišim ispitivanim koncentracijama akrilata (50%) utvrđen je veći procenat vijabilnih ćelija u slučaju zamene ekstrakata DMEM-om, kod svih tipova akrilata. Statistička značajnost postoji kod svih vrsta akrilata osim Simgal-a. U slučaju Triplex Cold-a ona je najvišeg nivoa ($p<0,001$).

Ekstrakti svih ispitivanih akrilata koncentracije 50% pokazali su umereni citotoksični efekat na vijabilnost *HeLa* ćelija.

Prilikom zamene ekstrakata akrilata koncentracije 5% medijumom, nije došlo do oporavka *HeLa* ćelija. Dobijeni rezultati za veće koncentracije ekstrakata pokazali su reverzibilnost nastalih promena kod Biocryl-a RN, Simgal-a i Triplex Cold-a. Efekat reverzibilnosti nije uočen kod Triplex Hot-a, osim za koncentraciju 50%.

Diskusija

Kako je protezna baza u kontaktu sa velikom površinom oralne sluzokože i u toku dužeg vremenskog perioda, postoji rizik za njeno oštećenje, bez obzira na uputstva proizvođača o njihovoj indiferentnosti. Zbog toga je ispitivanje bioloških osobina akrilata od izuzetnog značaja s obzirom na njihovu masovnu upotrebu u svakodnevnoj stomatoprotetskoj praksi. Pri tome, ispitivanje citotoksičnosti akrilatnih materijala u uslovima *in vitro* predstavlja veoma interesantnu oblast. U tom smislu, kontinuirane ćelijske kulture, kao biološki sistemi za testiranje, imaju značajnu ulogu iz više razloga. Ćelije je lako gajiti u kulturi, nema varijabilnosti koja bi se vezivala za različite donore tkiva a postoji i mogućnost ponavljanja testa pod identičnim uslovima [1,15].

U ovom radu ispitivan je efekat ekstrakata akrilata za bazu proteze na permanentnu ćelijsku liniju, *HeLa*. Ove ćelije su odabrane zbog njihove sličnosti sa epitelnim ćelijama oralne mukoze. Kako je protezna baza u kontaktu sa sluzokožom usne duplje efekti na ispitivanu ćelijsku kulturu mogu se smatrati klinički relevantnim. Zahvaljujući njihovoj izuzetnoj osetljivosti, eventualni hemijski uticaj akrilata na vijabilnost i gustinu ćelija mogao se precizno registrovati[16].

of Triplex Hot. Statistic significance exists only with Biocryl RN samples.

All acrylic resins, except Triplex Cold, showed a slight cytotoxic effect at concentration of 25%. Triplex Cold extracts revealed a moderate cytotoxic effect, which proves the fact that cold/polymerized acrylic resins are more toxic.

At the highest acrylic concentrations tested (50%), more viable cells were noticed an all acrylic resins' types, when the extracts were replaced by DMEM. Statistic significance exists with all acrylic resins, with the exception of Simgal. The highest level is in case of Triplex Cold ($p<0.001$).

The extracts of all tested acrylic resins with concentration of 50% showed moderate cytotoxic effect on *HeLa* cell viability.

While replacing 5% concentrated extracts of acrylic resins by a medium, *HeLa* cells' recovery did not occur. The obtained results for higher concentrations of extracts showed reversibility of the changes that occurred with Biocryl RN, Simgal and Triplex Cold. The effect of reversibility was not detected with Triplex Hot, except for 50% concentration.

Discussion

As the denture base is in long lasting contact with a large surface of oral mucous membrane, there is a risk of damaging, despite the manufacturers' instructions about the base' s indifference. Due to that, testing the biological features of acrylic resins is exterminaly important, considering their often use in everyday dental practice. In addition, cytotoxicity testing of the acrylic resins under in vitro conditions belongs to a very interesting researching field. In that context, continuing cell cultures, viewed as biological testing systems, play a significant role for various reasons. Cells are easy to be bred in a culture; there is no variability to be connected to different tissues donors; and there is a possibility of re-performing the test under identical conditions[1,15].

The study has examined the effect which the acrylic resins' extracts have on the permanent *HeLa* cell line. These cells were chosen for their similarity to the epithelial cells of the oral mucosa. As dentures are in contact with the mucous membrane of the oral cavity, the effects on the tested cell culture can be treated as clini-

Ispitivani materijali ispoljili su blagu do umerenu citotoksičnost, što je u saglasnosti sa nalazima Huang i sar. i Liu i sar[15,17]. Takođe su i nalazi Tsuchiya i sar., Cimpan i sar., Campancha i sar. ukazali na citotoksični efekat akrilata korišćenjem drugih *in vitro* testova [18,19,20]. Nasuprot ovim rezultatima, Jorge i sar. nisu utvrdili citotoksičnost akrilata[2]. Razlike u dobijenim rezultatima mogu se pripisati razlikama u eksperimentalnom dizajnu koje obuhvataju izabrani tip akrilata za testiranje, izbor ćelijske linije i primenu različitih testova za analizu citotoksičnosti.

U ovom radu, izmedju ostalog, utvrđivano je da li su nastale promene reverzibilnog karaktera, odnosno da li nakon prestanka delovanja toksičnih komponenti iz akrilata za bazu proteze dolazi do oporavka opservirane ćelijske linije. U smislu oporavka ćelija apoptoza (programirana ćelijska smrt) predstavlja kaskadni niz reakcija u kojem u pojedinim tačkama postoji mogućnost reverzije procesa i neophodni je sastavni deo ćelijske homeostaze za razliku od nekroze, koja je ireverzibilan proces i ukazuje na znatno manju kompatibilnost primenjenog materijala [21,22].

Buduća istraživanja mogla bi ići u pravcu određivanja mehanizma ćelijske smrti, a u cilju poboljšanja kvaliteta ovih materijala.

Zaključak

Sa porastom koncentracije ekstrakata ispitivanih materijala vijabilnost *HeLa* ćelija značajno opada i smanjije se mogućnost njihovog oporavka. Potpuni oporavak ćelijske kulture nakon zamene ekstrakata akrilata DMEM-om nije primećen ni u jednoj od ispitivanih koncentracija.

Zahvalnica

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cally relevant. Due to their extreme sensitivity, the possible chemical acrylic influence on the cell viability and density could have been precisely notified[16].

The tested materials demonstrated slight to moderate cytotoxicity, which corresponds with researches of Huang et al. and Liu et al.[15,17]. Also, the researches of Tsuchiya et al., Cimpan et al. and Campancha et al. pointed to the cytotoxic effect of acrylic resins, by doing *in vitro* experiments[18,19,20]. Contrary to these results, Jorge et al. didn't detect cytotoxicity of acrylic resins[2]. The differences in the obtained results may be contributed to the differences of the experimental design, and it includes: the acrylic resin type chosen to be tested, choice of cell line and application of different tests for analyzing cytotoxicity.

This study included, among other things, a research on whether the changes that appeared are reversible, i.e. whether the observed cell line recovers when the acrylic resins' toxic components stop working. Considered as cell recovery, apoptosis (programmed cell death) represents a successive chain of reactions in which there is possibility of a reversible process at some particular points. Unlike necrosis, which is irreversible and points to a significant shortage of compatibility of applied material, apoptosis is an essential part of cell homeostasis [21,22].

Researches in the future could have the direction of determining the mechanism of cell death. In the way, the quality of the acrylic materials could be important.

Conclusion

As the concentration of the acrylic resin extracts grows, the *HeLa* cell viability considerably diminishes and the possibility for their recovering is lessening. A compete recovery of the cell culture, after replacing the acrylic extracts by DMEM, hasn't been detected in any of the concentrations examined.

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