PREGLEDNI RAD
REVIEW ARTICLE

SAVREMENI ASPEKT ISPITIVANJA BIOKOMPATIBILNOSTI MATERIJALA U STOMATOLOGIJI

CONTEMPORARY ASPECT OF DENTAL MATERIALS BIOCOMPATIBILITY EXAMINATION TESTS

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KRATAK SADRŽAJ

Uvod.*Biokompatibilnost, kao esencijalna osobina materijala u stomatologiji, podrazumeva sposobnost materijala da nakon aplikacije obavlja određenu funkciju u organizmu ne izazivajući neželjeni odgovor tkiva domaćina. Testovi za ispitivanje biokompatibilnosti služe za detekciju sastojaka materijala koji bi mogli da dovedu do povrede ili oštećenja tkiva usne duplje i organizma uopšte.*
Cilj rada *bio je opis metoda koje se koriste za ispitivanje biokompatibilnosti materijala u stomatologiji.*
Materijal i metode. *Urađena je analiza publikovanih radova u periodu od 1990-2009. godine, i koji su indeksirana u Medline bazi podataka (US National Library of Medicine). Korišćene su ključne reči: testiranje, biokompatibilnost, stomatološki materijali.*
Rezultati. *U opisanom periodu na temu ispitivanja biokompatibilnosti različitih stomatoloških materijala objavljeno je 505 radova od kojih je 53 preglednih radova. 194 radova odnosilo se na in vitro, a 101 rad na in vivo istraživanja biokompatibilnosti.*
Zaključak. *Savremeni aspekt ispitivanja biokompatibilnosti stomatoloških materijala podrazumeva tri nivoa istraživanja: in vitro u laboratorijskim uslovima, in vivo testove na eksperimentalnim životinjama i testove primene. Klinička relevantnost rezultata dobijenih u uslovima in vitro je diskutabilna, ali se njima može znatno smanjiti broj testova na životinjama. Prava ocena o nekom materijalu može dati tek nakon dužeg korišćenja u kliničkoj praksi.*

***Ključne reči**: testiranje, biokompatibilnost, stomatološki materijali*

SUMMARY

Introduction. *As an essential feature of the materials in dental medicine, biocompatibility implies the ability of the material to perform a certain function after its application in an organism, without provoking a adverse response of the host tissue. Biocompatibility examination tests are used to detect the ingredients of the material which might possibly hurt or harm the mouth cavity tissue and the organism in general.*
The aim *of the work was to describe the methods used for examination of biocompatibility of materials in dentistry.*
Material and Methods. *This article reviews the literature published from 1990-2009., and indexed in the Medline Database (US National Library of Medicine). Used key words were testing, biocompatibility, dental materials.*
Results. *In this period, 505 manuscripts about the biocompatibility of different dental materials were published, 53 reviews. 194 of manuscripts were about in vitro biocompatibility tests, and 101 referred to in vivo examinations.*
Conclusion. *The contemporary aspect of examining biocompatibility of dental materials includes a three level research: in vitro tests in laboratory conditions, in vivo tests on experimental animals and tests of application. Clinical relevance of the results obtained under in vitro conditions is disputable, but these results can significantly reduce the number of experiments performed on animals. A proper evaluation of some material can be made only after a long enough use in clinical practice.*

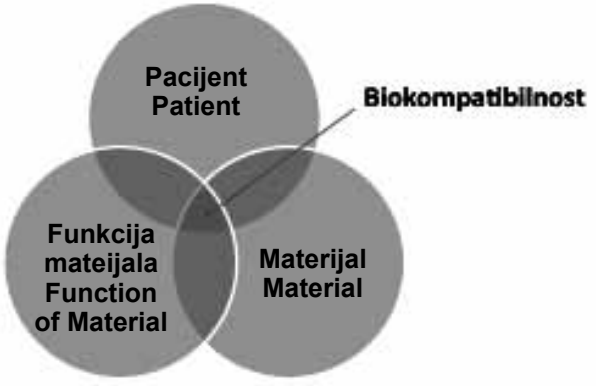
Key Words: test, biocompatibility, dental materials

Uvod

Biokompatibilnost se definiše kao sposobnost materijala da nakon aplikacije obavlja određenu funkciju u organizmu ne izazivajući neželjeni odgovor tkiva domaćina [1]. Biokompatibilnost podrazumeva harmoniju međusobne interakcije tkiva domaćina, korišćenog materijala i funkcije koju on obavlja, pri čemu se

Introduction

Biocompatibility is defined as an ability of a material to perform a certain function after its application in an organism, without provoking a adverse response of the host tissue[1]. Biocompatibility implies harmonious interactions between the host tissue, the material



Grafikon 1. Biokompatibilnost postoji samo u slučaju harmoničnog odnosa tkiva domaćina, materijala i funkcije koje on u organizmu obavlja

Graph. 1. Biocompatibility implies harmonious interactions between the host tissue, the material used and the function it performs.

reakcija tkiva domaćina zadržava u granicama tolerancije [2] (grafikon 1).

Odgovor organizma na prisustvo materijala je dinamičan proces, s obzirom da se telo menja starenjem ili pod uticajem bolesti. Vremenom nastaju i promene u samom materijalu, korozijom, zamorom, trošenjem [3]. Svaka od ovih promena može da promeni uslove koji su inicijalno davali odgovarajući i željeni biološki odgovor.

Uloga najvećeg broja stomatoloških materijala zasniva se na njihovim fizičko-mehaničkim osobinama [4]. Neželjena reakcija tkiva može biti posledica toksičnosti primenjenog materijala, ali i drugih faktora, kao što je akumulacija infektivnog materijala. Iako su reakcije tkiva na prisustvo stomatoloških materijala retke, ogroman broj svakodnevnih stomatoloških tretmana povećava mogućnost njihovog nastanka.

Osnovni zadatak testova za ispitivanje biokompatibilnosti jeste odstranjivanje svakog od sastojaka materijala koji bi mogao da dovede do povrede ili oštećenja tkiva usne duplje.

Cilj rada bio je opis metoda koje se koriste za ispitivanje biokompatibilnosti materijala u stomatologiji

Materijal i metode

Korišćena je literatura objavljena u periodu od 1990-2009. godine, indeksirana u Medline bazi podataka (*US National Library of Medicine*). Korišćene su ključne reči: testiranje, biokompatibilnost, stomatološki materijali.

used and the function it performs, as a result of which the host tissue reaction remains tolerable[2] (graph. 1).

The organism's response to the presence of the material is a dynamic process because of the fact that a body changes as it grows old or as it is affected by illnesses. In time, the material itself changes due to corrosion, fatigue, wasting [3]. Each of these changes can transform the conditions which initially gave an appropriate and desirable biological response.

The majority of dental materials have a role based on their physical-mechanical features[4]. A negative tissue reaction can be a consequence of toxicity of the applied material, but of some other factors as well, such as accumulation of infective material. Although tissue reactions to the dental material presence are rare, a huge number of everyday dental treatments increase the possibility of them to appear.

The main task of the biocompatibility examination tests is to remove every ingredient from the material which could possibly hurt or damage the mouth cavity tissue.

The aim of the work was to describe the methods used for examination of material biocompatibility in dental medicine.

Material and Methods

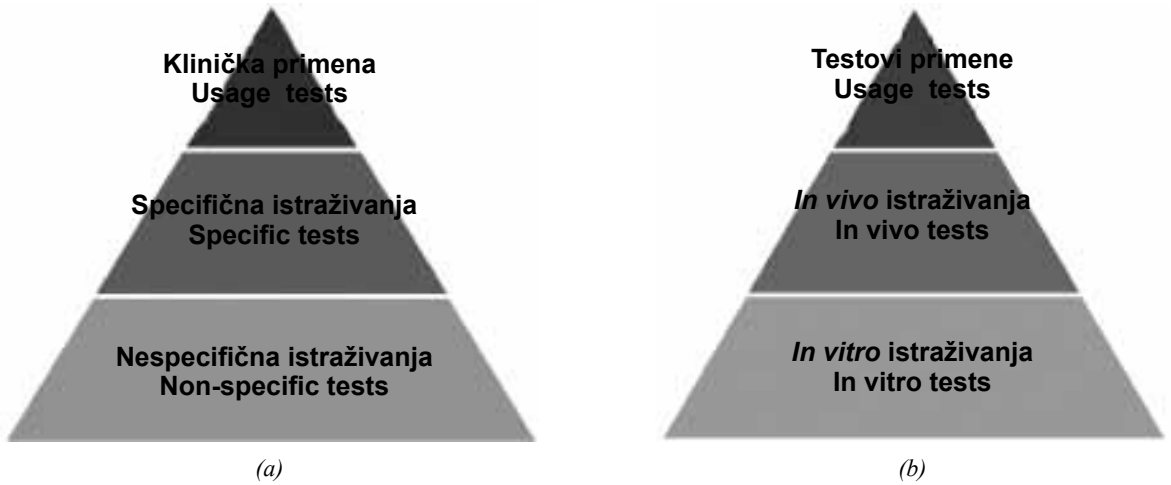
This article is based on literature published from 1990-2009., and indexed in the Medline Database *(US National Library of Medicine)*. Used key words were testing, biocompatibility, dental materials.

Results

In period 1990-2009, 505 manuscripts about the biocompatibility of different dental materials were published and indexed in the Medline Database, 53 reviews. 194 of manuscripts were about *in vitro* biocompatibility tests, and 101 referred to *in vivo* examinations.

Dental materials biocompatibility examination tests

Biocompatibility tests are performed under *in vitro* and *in vivo* conditions. The contemporary aspect of examining bio-



Grafikon 2. (a) i (b) Savremeni koncept ispitivanja biokompatibilnosti materijala u stomatologiji

Graph. 2. (a) i (b) Contemporary aspect of dental materials biocompatibility examination tests.

Rezultati

U periodu na temu ispitivanja biokompatibilnosti različitih stomatoloških materijala objavljeno je 505 radova i indeksirano u Medline bazi podataka, od kojih 53 preglednih. 194 radova odnosilo se na *in vitro*, a 101 rad na *in vivo* istraživanja biokompatibilnosti.

Testovi za ispitivanje biokompatibilnosti stomatoloških materijala

Testovi biokompatibilnosti se realizuju u *in vitro* i *in vivo* uslovima.

Savremeni aspekt ispitivanja biokompatibilnosti stomatoloških materijala podrazumeva tri nivoa istraživanja. Prvi stepen se odnosi na testiranje materijala u laboratorijskim uslovima na kulturama ćelija, tkiva ili organa. Sledeći nivo predstavljaju eksperimenti na životinjama, sa ciljem da se simuliraju uslovi u organizmu čoveka. Treći nivo su različiti testovi primene materijala u kliničkim uslovima (grafikon 2).

Glavni cilj standardizacije procedura ispitivanja biokompatibilnosti jeste poboljšanje reproduktivnosti i mogućnost ponavljanja testova, kao i lakša komparacija rezultata dobijenih u različitim laboratorijama. Internacionalni standardi pokrivaju specifične stomatološke materijale (ISO/DIS 7405) i medicinska sredstva (ISO 10993), koja uključuju i materijale u stomatologiji. Nestandardni testovi služe za vrlo specifične naučne probleme [5,6].

***In vitro* testovi** biokompatibilnosti obavljaju se van živog organizma s ciljem da simuliraju biološku reakciju tkiva koje dolazi u kontakt

compatibility of dental materials includes a three level research. The first step relates to material testing in lab conditions using cell, tissue or organ cultures. The next step is experiments on animals, with the aim of stimulating the conditions of a human body. The third level consists of different tests of material application in clinical conditions (graph. 2).

The major goal of standardization of biocompatibility examination procedures is to improve reproduction and the possibility of re-performing tests, as well as easier comparison of results obtained in different laboratories. International standards cover specific dental materials (ISO/DIS 7405) and medical means (ISO 10993), which include materials in dental medicine, too. Non standard tests are used for very specific scientific issues[5, 6].

***In vitro* tests** of biocompatibility are performed outside organism with the aim of stimulating a biological reaction of a tissue that comes in contact with the examined material[7]. An initial review of biocompatibility of dental materials is quite often made by using tests of cytotoxicity (table 1). Under *in vitro* conditions, mutual influences of biomaterials and microorganisms in the patient's mouth can be examined[8, 9].

The elements of *in vitro* tests are: a biological system, a contact type of the material and the examined system, and a final biological reaction to the presence of the material[4, 10].

The examined *biological systems* can be: tissue and organ cultures, cell cultures and organelles. What is usually used for *in vitro* ex-

Tabela 1. Kratak opis različitih metoda koje se koriste za testiranje citotoksičnosti materijala u stomatologiji

Table 1. Summary of different assays used in cytotoxicity tests of dental materials.

Biološka proba / Biological assay	Mehanizam / Mechanism	Vrsta probe / Test type	
MTT	promene aktivnosti mitohondrijalne dehidrogenaze / mitochondrial dehydrogenase activity	Kolorimetrijki testovi Colorimetric assays	Testovi vijabilnosti i proliferacije Cell viability and proliferation assays
LDH	promene aktivnosti laktat dehidrogenaze / lactate dehydrogenase activity		
Almar plavo / Almar blue	hemijska redukcija hranjivog medijuma / chemical reduction of culture medium		
Neutral crveno/ Neutral red	oštećenje ćelijske membrane (bojenje vitalnih ćelija) / membrane damage (stains vital cells)		
Propidijum jodid	oštećenje ćelijske membrane (bojenje mrtvih ćelija) / membrane damage (stains dead cells)		
Tripan plavo / Tripan blue	oštećenje ćelijske membrane (bojenje mrtvih ćelija) / membrane damage (stains dead cells)		
BrdU	inkorporacija u novosintetisanu DNK / incorporation into newly synthesized DNA	Testovi inkorporacije Incorporation assays	
^{3}H-timidin	inkorporacija u novosintetisanu DNK / incorporation into newly synthesized DNA		
Merenje sadržaja proteina / Protein content	proliferacija i specifična bojenja / proliferation and specific stains	Merenja količine intracelularnih supstanci Intracellular content measurement	
Merenje sadržaja DNK / DNA measurement	proliferacija i specifična bojenja / proliferation and specific stains		
Markeri inflamacije / Inflammatory markers	merenje količine protein indikatora inflamacije / inflammation indicators	Ćelijske funkcije Assays based on cell function	
Determinacija glutationa (GSH) / Glutathione determination (GSH)	indikator toksičnosti / toxicity indicator		
HSP	indikator ćelijskog stresa / stress indicator		
Tip ćelijske smrti / Apoptosis	specifični marker apoptotične odnosno nekrotične smrti / type of cell injury and death		

sa ispitivanim materijalom [7]. Inicijalno sagledavanje biokompatibilnosti stomatoloških materijala najčešće se obavlja kroz testove citotoksičnosti (tabela 1). U uslovima *in vitro* mogu se ispitivati i međusobni uticaji biomaterijala i mikroorganizama sa kojima su u kontaktu u ustima pacijenta [8,9].

Komponente *in vitro* testova su biološki sistem, vrsta kontakta materijala i ispitivanog sistema i konačna biološka reakcija na prisustvo materijala [4,10].

Ispitivani *biološki sistemi* mogu biti: kulture tkiva i organa, ćelijske kulture i organele. Za *in vitro* ispitivanja toksičnosti stomatoloških materijala obično se koriste jednoslojne kulture ćelija i trodimenzionalni (3D) modeli oralne mukoze dobijeni tkivnim inžinjeringom [4]. 3D modeli podrazumevaju jednu ili više ćelijskih linija kultivisanih u specijalnim najlonskim vrećicama, čiji izbor i raspored verno simuliraju *in vivo* uslove. Izbor ćelija (permanentne i primarne ćelijske linije) zavisi od biološkog cilja predviđenog u testu citotoksičnosti.

Permanentne (kontinuirane, inmortalne) ćelijske linije su jasno morfološki i funkcionalno definisane, relativno se lako kultivišu i komercijalno su dostupne. Njihova upotreba omogućava dobru korelaciju rezultata različitih laboratorija. Sa druge strane, one su jednostavniji replikacioni sistemi, bez specifičnog metaboličkog potencijala normalno prisutnog u organizmu. U cilju ispitivanja potencijalne toksičnosti stomatoloških materijala najčešće se koriste fibroblastne, epitelne, osteoblastne, osteoklastne, odontoblastne i tumorske ćelije. Tumorske ili transformisane ćelijske linije, zbog promena u genomu, mogu dati u izvesnoj meri promenjen odgovor ćelijske kulture na prisustvo ispitivanog materijala u odnosu na netransformisane, diploidne ćelije [11,12].

Primarne ćelijske linije dobijene su biopsijom oralne sluzokože, pulpe, parodonta ili iz periferne krvi i ustanovljene su u samoj laboratoriji. Njima se postiže bolja simulacija situacije *in vivo* jer poseduju sve osobine tkiva iz koga su uzete. Izbor primarnih ćelija zavisi od vrste ispitivanog materijala, kao i od vrste tkiva sa kojim je ispitivani materijal u neposrednom kontaktu [13]. Dostupne su samo u specijalizovanim laboratorijama, te se koriste za specifična naučna istraživanja [4].

Uvođenje 3D modela imalo je za cilj prevazilaženje glavnog nedostatka jednoslojnih ćelijskih kultura, kliničke nerelevantnosti. Upotrebom ovih modela moguće su složene analize biološkog odgovora oralne mukoze na prisustvo restaurativnog materijala, čime se smanjuje amination of dental materials toxicity are single layer cell cultures and 3D models of oral mycosis obtained by tissue engineering[4]. 3D models imply a single or multi cell lines cultivated in special nylon meshes, whose choice and order stimulate *in vivo* conditions. The choice of cells (permanent and primary cell lines) depends on the biological goal anticipated in the test of cytotoxicity.

Permanent (continuing, immortal) cell lines are clearly defined both morphologically and functionally, they can be cultivated relatively easy and are commercially available. Their use enables efficient correlation of results between different laboratories. On the other side, they represent simpler replication system, without the specific metabolic potential which is normally present in an organism. For examination of potential toxicity of dental materials, fibroblast, epithelial, osteoblast, odontoblast and tumor cells are most frequently used. Due to changes in the genome, tumor or transformed cell lines may produce a moderately different response of the cell culture to the presence of the examined material, when compared to non-transformed diploid cells[11, 12].

Primary cell lines are obtained by biopsy of oral mucous membrane, the pulp, the parodont or from the peripheral blood, and are defined in the lab itself. Using primary cell lines, a better simulation of *in vivo* situation is achieved because they possess all the characteristics of the tissue they have been taken out of. The choice of primary cells depends on the type of the examined material, as well as on the type of the tissue that the examined material is in direct contact with[13]. They are available only in specialized laboratories and are used for specific scientific researches[4].

Introducing 3D models was with the aim of overcoming the major defect of single layer cell lines, and that is clinical irrelevance. Using these models enables complicated analyses of oral mycosis' biological response to the presence of restoration material, decreasing the need of performing tests on animals in this way[10].

The contact between the cell culture and the examined material is achieved directly, indirectly or through an extract as a mediator[8].

When the contact is direct, cytotoxicity is measured by a cell death rate in the function of time exposition and distance of the hard sample[14]. The most frequent complications of

Tabela 2. Specifične metode za ispitivanje biokompatibilnosti restaurativnih stomatoloških materijala

Table 2. Specific methods of dental restorative material's examinations

IN VITRO ISTRAŽIVANJA IN VITRO EXAMINATIONS	**Test sa jednoslojnom ćelijskom kulturom / Monolayer cell culture test**	**humane i animalne ćelije pulpe, humani THP-1 monociti, MDPC-23 odontoblasti miša, L-929 fibroblasti miša i dr. / human and animal pulp cells, human THP-1 monocytes, MDPC-23 mouse odontoblasts, L-929 mouse fibroblasts etc.**
	Test dentinske barijere / Dentin barrier test	**dentinske ploče koje u specijalnim komorama razdvajaju ispitivani uzorak i ćelijsku kulturu / cell culture and test material are separated by the dentin disc**
	Test mukozne barijere / Mucosal barrier test	**kultura humanih fibroblasta u 3D modelu-najlonskoj mrežici obloženoj slojem epitelnih ćelija / human fibroblasts which are grown in 3D nylon mesh covered with epithelium cells**
IN VIVO ISTRAŽIVANJA IN VIVO EXAMINATIONS	**Pulpni i dentinski test / Pulpal and dentinal testing**	**ispitivanja stomatoloških restaurativnih materijala na životinjama i pacijentima volonterima / experimental researches of dental restorative materials on animals or patients volunteers**
	Test prekrivanja pulpe / Test of pulpal covering	
	Endodontski test / Endodontic test	**ispitivanja stomatoloških restaurativnih materijala na životinjama / experimental researches of dental restorative materials on animals**

je potreba za testiranjem na eksperimentalnim životinjama [10].

Kontakt između ćelijske kulture i ispitivanog materijala ostvaruje se direktnim i indirektnim putem i postredstvom ekstrakata [8].

U slučaju direktnog kontakta, citotoksičnost se meri stopom ćelijske smrti u funkciji vremena ekspozicije i udaljenosti od uzorka koji je u čvrstom agregatnom stanju [14]. Najčešće komplikacije *in vitro* testova sa direktnim kontaktom su bakterijska kontaminacija kulture testiranim materijalom jer se sterilizacijom stomatološkog materijala menjaju i njegove osobine, kao i mehaničko oštećenje ćelija neposrednim dodirom sa materijalom [4,10].

Indirektni kontakt ćelija i materijala podrazumeva upotrebu propustljivog intermedijuma, najčešće sintetičkog filtera ili dentinske membrane. Ovaj metod je nezavistan od fizičkog stanja materijala, te on može biti u čvrstom, polutečnom ili tečnom stanju. Intimni kontakt između materijala i ćelija omogućava dejstvo i komponenti koje nisu rastvorljive u vodi [14].

in vitro tests with direct contact are: bacterial contamination of the cell culture with the tested material and mechanical damage of the cells by immediate contact with the material. These complications appear because the sterilization of the dental material causes changes in its characteristics[4, 10].

Indirect contact between cells and the material implies the use of porous intermedium, most frequently synthetic filter or dentine membrane. This method does not depend on whether the material is hard, semi-liquid or liquid. The contact between the material and the cells enables a reaction even of the components not soluble in water[14].

A contact can be set up between dissoluble materials and cells by extracting released components using a solvent (cell medium, distilled water, physiological solution and puferized sodium chloride solutions)[7]. Sterilization of the solvent is done by centrifuging process or by the process of sterile filtration, which

Kontakt između nerastvorljivih materijala i ćelija može se uspostaviti ekstrahovanjem oslobođenih komponenti rastvaračem (ćelijski medijum, destilovana voda, fiziološki rastvor i puferisani rastvori soli) [7]. Sterilizacija rastvarača vrši se centrifugiranjem ili sterilnim filtriranjem, čime se značajno smanjuje mogućnost kontaminacije. Ovo je najčešće primenjivana metoda ispitivanja citotoksičnosti različitih stomatoloških materijala (restaurativni materijli, dentalni cementi, amalgami, akrilati za bazu zubne proteze i dentalni adhezivi) [15, 16, 17].

Biološka reakcija ćelija može da se opiše morfološki ili kvanititativnom analizom. Za jednostavnija istraživanja potencijalne toksičnosti stomatoloških materijala koriste se metode koje se baziraju na oštećenju ćelijske membrane i ispitivanju stepena ćelijske vijabilnosti i proliferacije (agar i filter difuzioni test, almar plavo, tripan plavo, neutral crveno, LDH i MTT test, propidijum jodid test, testovi inkorporacije radioizotopa, kao što su ^{3}H-timidin i bromo dezoksouridin (BrdU) i dr.). U cilju istraživanja mehanizma toksične aktivnosti ispitivanog materijala potreban je specifičniji metod istraživanja, pa se koriste komplikovaniji testovi bazirani na ćelijskim funkcijama (merenje količine proteina zapaljenja i proteina indikatora ćelijskog stresa Heat-Shock proteina (HSPs), test determinacije glutationa, određivanje tipa ćelijske smrti i dr.) [18,19].

Prednost *in vitro* testova je mogućnost ponavljanja pod identičnim uslovima, stroga kontrola po svakom parametru, laka izvodljivost i ekonomičnost [20,21]. Sa druge strane, u *in vitro* za razliku od *in vivo* uslova nedostaju odbrambene reakcije tkiva na prisustvo ispitivanog materijala [22]. Rezultati *in vitro* testova se, pre svega, odnose na akutni toksični efekat. Iz tih razloga da se interpretiraju samo unutar serije blisko povezanih materijala (relativna toksična analiza) [23].

***In vivo* testovi** biokompatibilnosti podrazumevaju eksperimentalna istraživanja na životinjama ili na pacijentima koji su dobrovoljno pristali na tu saradnju (zubi indikovani za ekstrakciju iz ortodontskih razloga).

Eksperimentalna istraživanja biokompatibilnosti na malim laboratorijskim životinjama vrše se testovima implantacije, alergijskim testovima i ispitivanjima akutne i hronične sistemske toksičnosti stomatološkog materijala [9].

Efikasnost testova na životinjama veća je u odnosu na *in vitro* istraživanja. Sa druge strane, ovi testovi su skupi, zavisni od vremena, teško je pratiti kontrolne varijabile, a samo

significantly decreases the possibility of contamination. This is the most frequently applied method of examining cytotoxicity of different dental materials (restoration materials, dental cements, amalgams, denture base acrylates and dental adhesives)[15, 16, 17].

Biological cell reaction can be described either morphologically or by a quantative analysis. For less complicated researches of dental materials' potential toxicity, methods are used which are based on cell membrane damage and examining the level of cell viability and proliferation (agar and filter diffusion test, almar blue, tripan blue, neutral red, LDH and MTT tests, propidium iodide test, test of radioisotope incorporation, such as ^{3}H-timidine and bromodeoxyuridine (BrdU) etc.). For researching mechanisms of toxic activity of the examined material, a more specific method of research is needed. Thus, more complicated tests are used based on cell functions (measuring of the amount of inflammation protein and the Heat Shock protein (HSPs), glutathione determination test, determination of the cell death type etc.)[18,19] (table 2).

The advantages of *in vitro* tests are: the possibility of re-performing them under identical conditions, strict control of every parameter, easy performance and the economical aspect[20, 21]. On the other hand, when compared to *in vivo* conditions, *in vitro* tests lack defensive reactions of tissue to the presence of the examined material[22]. The results of *in vitro* tests are, basically, related to the acute toxic effect. Therefore, they are interpreted only inside a series of closely connected materials (relative toxic analysis)[23].

***In vivo* tests** of biocompatibility imply experimental researches on animals or patients who voluntarily agreed to that (teeth indicated for extraction for orthodontic reasons).

Experimental researches of biocompatibility on animals are performed by using tests of implantation, allergy tests and examination of acute and chronic systematic toxicity of dental material[9].

The efficiency is greater with the tests on animals than with the *in vitro* researches. On the other side, these tests are expensive, time dependant, it is difficult to follow the controlling variables, and the use of animals for experimental purposes is an ethical issue. Apart from that, morphological and functional characteristics of human and animal tissues and organs are

korišćenje životinja u eksperimentalne svrhe je etički probem. Pored toga, postoje razlike u morfološkim i funkcionalnim osobinama tkiva i organa ljudi i životinja, te stoga dobijene rezultate treba uzimati sa rezervom. Najbolji animalni modeli su humani primati, ali se oni najređe koriste u ekperimentalnim istraživanjima. Moderni koncept testiranja biokompatibilnosti stomatoloških materijala zasnovan je na reduciji eksperimenata na životinjama kroz bolju simulaciju *in vivo* uslova u *in vitro* eksperimentima [24] (tabela 2).

Testovi primene su specijalna klinička kontrola novog materijala na volonterima i predstavljaju najrelevantnije testove biokompatibilnosti. Skupi su, vremenski ograničeni, veoma teški za kontrolu i interpretiranje rezultata i pravno i etički jako komplikovani [2].

Klinička relevantnost rezultata dobijenih u uslovima *in vitro* je diskutabilna. Kliničko iskustvo pokazuje da cink oksid eugenol cement nije toksičan za pulpu, dok subkutana implantacija na životinjama dovodi do značajne inflamacije okolnog kiva. Testovi *in vitro* pokazali su da ovaj cement može biti veoma toksičan [24].

Zaključak

S obzirom na sve veći pritisak javnosti u cilju zaštite eksperimentalnih životinja i kako ispitivanje na ljudima podleže strogim etičkim principima, prednost treba dati testovima zasnovanim na veštačkim modelima tkiva i organa, sa što boljom simulacijom uslova koji postoje u živom organizmu. Neophodno je da svakom *in vivo* prethodi detaljna analiza na kompjuterski simuliranim modelima i ispitivanje u uslovima *in vitro*.

Prava ocena o nekom materijalu može dati tek nakon dužeg korišćenja u kliničkoj praksi.

different, so, the obtained results should not be taken for granted. The best animal models are the primates, but they are used in experimental researches very rarely. The modern concept of biocompatibility testing of dental materials is based on the reduction of experiments on animals by better simulation of *in vivo* conditions *in vitro* experiments[24].

Usage tests represent a special clinical control of new material on volunteers, and they are the most relevant type of biocompatibility tests. They are expensive, limited in time, very difficult to be controlled, and the interpretation of the results is rather complicated, both legally and ethically[2].

Clinical relevance of the results obtained under *in vitro* conditions is disputable. Clinical practice shows that zinc oxide eugenol cement is not toxic for the pulp, unlike the subcutaneous implantation on animals, which causes a significant inflammation of the surrounding tissue. *In vitro* tests showed that this cement type can be very toxic[24].

Conclusion

The public pressure for protection of experimental animals and strict ethical principles about experiments on people lead to a conclusion that priority should be given to tests based on artificial models of tissues and organs, with as better as possible simulation of conditions existing in a live organisms. It is necessary that every *in vivo* is preceded by a detailed analysis on a computer simulated models and by examination under *in vitro* conditions.

A proper evaluation of some material can be formed only after a long enough use in clinical practice.

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