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Contents / Sadržaj

Bolje je znati, nego naopako i zlo znati.
Dositej Obradović

Kako živimo u vremenu gde je „naopako" znanje naša svakodnevica, ovaj urednički komentar sam zato i započeo sjajnim citatom velikog Dositeja, koji je na to ukazao još mnogo ranije.

Ideju za ovaj urednički komentar dobio sam u razgovoru s jednim prijateljem u neformalnoj priči o vremenu i trenutku čiji smo saučesnici. Naravno, kako je politika postala sastavni deo naših života, pa i nauke, i ova priča je vezana za one koji nam, nažalost, oblikuju svakodnevicu.

Naime, jedan aktivni društveni radnik (čitaj političar) poželeo je da bude direktor jedne vrlo važne i visoke državne institucije. A dobro je poznato da je razmeštanje „podobnih" i poslušnih na važna i odgovorna mesta naša društvena stvarnost. Kako je za to mesto bilo zainteresovano više „kvalitetnih" kadrova, želja nije mogla biti ostvarena. Zato mu je ponuđeno mesto direktora jedne univerzitetske naučne ustanove. Nažalost, ni ovde nije imao „sreće" jer je i sam uvideo da mu kompetencije nisu zadovoljavajuće s obzirom na srednjoškolsko obrazovanje.

Mnogi koji ovo čitaju pomislili bi da je ovo priča iz nekog pozorišnog komada, neke satire ili možda romana iz 18. ili 19. veka. Ali, nažalost, nije. Ova priča je ipak „neuobičajena" jer ovaj „podobni" kadar nije dobio posao. E, to je već neuobičajeno, jer je činjenica da su obrazovani, odgovorni i učeni uglavnom „nepodobni" i ne mogu doći na mesta za koja su kompetentni. Oni bi, naime, svojim znanjem i profesionalnim pristupom mogli zasmetati svekolikom „napretku" i ugroziti „kompetentnije" kadrove, jer struka i znanje kao kriterijumi su danas uglavnom na podu, a „podobnost" i poslušnost na vrhu u svim segmentima života, uključujući i naučne i univerzitetske ustanove.

Zato je lako objasniti sunovrat u nauci, obrazovanju, kulturi, zdravstvu i društvu uopšte. O svemu odlučuju uglavnom neuki, korumpirani, bahati i oni sa kupljenim diplomama. Oni, nažalost, kreiraju svaki segment našeg života, a njihov odnos prema mnogim društvenim problemima i pojavama je uglavnom ličan.

Rešavanje problema i planiranje najčešće zavise od njihovog ličnog poimanja i osećaja i nauke i kulture i zdravlja i ekonomije, ali i svega onog što im omogućava mesto ili funkcija. A njihov lični odnos prema svemu društveno važnom je prilično skroman, inferioran i neodgovoran, jer uglavnom zavisi od njihove „teško stečene diplome".

Zato u vremenima gde politička funkcija nudi svekolike kompetencije dolazi do brojnih paradoksa. Tako je moguće da kulturom upravljaju nedovoljno vaspitani i bahati, zdravstvom netalentovani i neiskusni, prosvetom neobrazovani, medijima politički lojalni koji bestidno sebe nazivaju novinarima.

Iako je stručnost i kompetentnost javna, lako proverljiva i dostupna (jer je to lična karta svake obrazovane i odgovorne osobe), u vremenu u kojem živimo mnogo je važnija formalna kompetentnost (čitaj podobnost). Zahvaljujući takvoj „kompetentnosti" i narcisoidnoj potrebi da se svaki postupak naširoko objašnjava, bez ikakvih principa se sahranjuje etika, čast i dostojanstvo, a škola, obrazovanje i istina izopštava iz normalnog života.

Urednički komentar ću završiti citatom Vladike Velimirovića: „Mudar i lud hodaju istim putem pod suncem, no bacaju senke na dve suprotne strane". Nadam se da je ipak lako odvojiti istinu od propagande i lažne diplome i plagirane doktorate od istinskog naučnog znanja i suštinskih ljudskih kvaliteta.

Prof. dr Slavoljub Živković

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Forensic Dentistry – the key to the truth

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SUMMARY

Introduction Human organism can be identified through testing and analysis of DNA sequences. The most common source of DNA for analysis is blood, soft tissues, hair, bones and teeth. Teeth represent a tissue of choice for analysis in those cases where there is high degree of degradation of other tissues. Hard tooth structure provides protection and preservation of DNA molecules.

The aim was to investigate which group of teeth and dental tissue (pulp or hard dental tissues) has the greatest amount of DNA.

Material and method Forty-five extracted teeth were analyzed. In the first examination 30 teeth were divided into the three groups (10 teeth each): first group were incisors, second premolars and third molars. The teeth were measured before and after the procedure of DNA isolation using special scale with precision of 0.02-0.000005ng. The procedure included grinding teeth in a blender and DNA isolation using commercial kits (isolation with magnetic particles). For the second test 15 teeth divided into two groups were used. In the first group isolation of DNA molecules was performed from pulp tissue, and in the second group from hard dental tissues. The quantification of samples was done with Quantifiler® Duo DNA Quantification Kit by Applied Biosystems.

Results The greatest amount of DNA was obtained from molars (0.230011ng/µl/g) while the smallest amount of DNA was obtained from incisors and it was 0.06437ng/µl/g. In addition, the amount of DNA isolated from pulp tissue was significantly greater than that from hard dental tissues (pulp of molars obtained quantitatively the largest amount of DNA).

Conclusion Main tissue to be used for the isolation of DNA from a tooth is pulp, but in those cases when it is not present (endodontic treatment), hard dental tissues provide sufficient quantity of DNA for identification procedures. The group of teeth that provides the greatest amount of DNA is molars.

Keywords: DNA isolation; molars; dento-pulp complex

INTRODUCTION

Forensic dentistry represents a scientific discipline in which field of interest is the tooth and all its features, starting with its morphology and analysis of its structural components, in order to get information to solve criminal cases or use in court (legal) system. Forensic odontology or Forensic dentistry is defined by Keiser-Neilson in 1970 [1] as "the branch of forensic medicine that in the interest of legal system handles and examines dental evidence and appropriately evaluates and presents dental findings". The most common role of forensic dentist is identification of deceased individuals [2]. Dental identification includes two modes. The first one is comparative identification used to verify that the remains of a deceased person and the person represented by dental data obtained before death (antemortem) are one and the same individual. The second method includes cases where data before death (antemortem) does not exist. In that case, forensic dentist complete data after death (postmortem) obtaining the deceased person's individual characteristics in order to compare them with antemortem data. This process is called postmortem dental profiling.

Human body can also be identified through testing and analysis of DNA sequences. Each cell of an individual contains multiple DNA copies. Every human being is characterized by a unique (single) DNA sequence due to hypervariable regions of DNA that are specific for that person. The sequence of DNA molecules called base pairs (bp) in each individual varies with the exception of identical twins and is called sequence polymorphism. Length polymorphism as AATG-AATG (repeat twice), or AATG-AATG-AATG (triple repetition), called Short Tandem Repeats (STR) is used in forensic identification [3, 4]. These repetitive sequences are called DNA fingerprints and the procedure for their identification is called DNA typifying, i.e. profiling (DNA Profiling). DNA profiling represents standard procedure in forensic system for human identification, criminal cases and determining fatherhood [5].

Beside DNA isolation from hair, bones, dried soft tissue, teeth give best usable DNA for identification because of the hard dental tissue that physically protects the pulp. Most often tissue used for the isolation of DNA is pulp tissue, which is connective tissue composed of cells (odontoblasts, fibroblasts, endothelial cells, peripheral nerves, undefined mesenchymal cells, macrophages and

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lymphocytes). However, in cases of endodontically treated tooth, where pulp is removed, DNA may be extracted from calcified tissue, dentin or dentin and cement together by the milling procedure to produce powder. DNA isolation is also possible from periodontal fibers and attached periodontal bone fragments.

The quality and quantity of extracted DNA from teeth depends on several external and individual factors. From individual factors, special attention is given to the volume of pulp chamber that is different in different teeth groups. Like for other tissues, DNA isolation from teeth depends on external influences like temperature, time of death, humidity, bacterial and fungal contamination [6, 7]. Also, the fastest DNA molecule degradation occurs in the first two years, depending on external and personal factors [6].

The aim was to investigate which group of teeth and dental tissue (pulp or hard dental tissues) has the greatest amount of DNA.

MATERIAL AND METHODS

Forty-five extracted teeth without major caries damage (*in vitro* study) extracted at the Clinic for Oral Surgery at the University Dental Clinical Center-St.Pantelejmon in Skopje were analyzed. DNA analyses were conducted at the Institute of forensic medicine, Criminology and Medical deontology. The method with magnetic particles as solid phase for binding the DNA molecule was used (Prepfiler Kit from ABI ™).

This technique of DNA isolation (unlike the old process of organic isolation) is based on solid phase extraction (SPE). It uses columns filled with silica (or other solid phase) that selectively bind (adsorb) nucleic acids (DNA or RNA) depending on the environmental pH and concentration of salt in the buffer (in our case the solid phase were magnetic particles). The columns and all buffers used to perform the procedure come in the form of commercial kits and the procedure was performed according to the manufacturer's instructions.

Quantification of samples was performed using the Quantifiler DNA Quantification Kit (Applied Biosystems) on 7500 Real Time PCR System. This approach allows DNA quantification in the initial sample by measuring the fluorescence signals during amplification of the targeted sequence. The primary objective of the Real Time PCR quantification is determining "amplifiable" DNA present in the sample. The input amount of Quantifiler® Duo Primer Mix for quantification of one sample was 10.5 µl, and the input amount of Quantifiler® Duo PCR Reaction Mix for quantification of one sample was 12.5 µl. The volume of a sample added into the reaction for quantification was 2 µl. The full volume of a reaction mix was 25 µl.

The first examination comprised 30 teeth divided into the three groups of 10 teeth: incisors, premolars and molars. The procedure of DNA isolation from teeth included teeth grinding in a blender and use of commercialized kits (isolation with magnetic particles). With this method, teeth that had previously done endodontic treatment could not be used for DNA isolation [8]. First, the teeth were rinsed under the tap water and surface was well cleaned with a scalpel and a brush. In addition, the teeth were kept in ultrasonic bath in 1% sodium hypochlorite (NaOCl) twice for 10 minutes with replacement of NaOCl solution. This procedure was repeated also twice with distilled water. The cleaning procedure took about 40 minutes. The teeth were placed then in a fume cupboard to dry overnight. Dried teeth were weight first and then ground in a blender for small bones and teeth to obtain tooth powder.

For the second test 15 teeth divided into two groups were used. In the first group isolation of DNA molecules was performed from the pulp tissue, and in the second group the same teeth that pulp was collected from were grinded and used for DNA isolation. Pulp was collected using nerve extirpators after performed horizontal sectioning of the tooth. This procedure of horizontal separation of the tooth in the line of the neck of the tooth was done in order to preserve occlusal surface of the tooth that can serve for further procedure of identification. This separation was done using diamond burs and micro motor. The bur was moved around the teeth in circular motions. When close to pulp chamber, teeth were split with lever, to avoid overheating and mechanical damage of pulp tissue, and thus contamination of the material.

RESULTS

In our first test the analysis and comparison of different groups of teeth, freshly extracted incisors, premolars and molars was done. Those are three groups differing in size and number of roots, i.e. there are large morphological differences between them. The results are shown in Tables 1-3.

In the second test the amount of DNA obtained from the pulp and hard dental tissues was compared. The results are shown in the Tables 4-5.

DISCUSSION

In forensic cases of identification, where human remains are extremely damaged or degraded, teeth and bones are often the only source of DNA [9, 10]. Genetic analysis of teeth depends on the amount of DNA present, the level of degradation and efficiency of the collecting processes of DNA and methods used for DNA isolation [11]. With the current knowledge about the structure of teeth and after death degradation of DNA in dental tissue, our paper offers optimal selection of teeth, in order to increase the effectiveness of DNA extraction and DNA profiling.

It can be seen that the greatest amount of DNA was obtained is in the group three or group of molars and it was 0.230011 ng/µl/g tooth, while the smallest amount of isolated DNA was obtained in the first group, i.e. the incisors and it was 0.06437 ng/µl/g tooth. The concentration of isolated DNA from the group of molars was four times greater than group of premolar and five times than group of incisors. Comparison of the first and second group, i.e. group of premolars and incisors indicated

Table 1. Quantity of isolated DNA from solid dental tissue from group of incisors
Tabela 1. Količina izolovane DNK iz čvrstog zubnog tkiva iz grupe sekutića

Amount of DNA isolated from the dental tissue Količina DNK izolovana iz zubnog tkiva				
	Weight of the tooth (g) Težina zuba (g)	Type of the tooth Vrsta zuba	DNA concentration (ng/µl) Koncentracija DNK (ng/µl)	DNA concentration per gram of the tooth (ng/µl/g) Koncentracija DNK po gramu zuba (ng/µl/g)
1.	0.52	Incisor Sekutić	0.0160	0.030769
2.	0.29	Incisor Sekutić	0.0451	0.155517
3.	1.33	Incisor Sekutić	0.0124	0.009323
4.	1.24	Incisor Sekutić	0.0585	0.047177
5.	0.10	Incisor Sekutić	0.00216	0.0216
6.	0.57	Incisor Sekutić	0.0210	0.036842
7.	1.01	Incisor Sekutić	0.0531	0.052574
8.	0.13	Incisor Sekutić	0.0224	0.172307
9.	0.62	Incisor Sekutić	0.0635	0.102419
10.	0.24	Incisor Sekutić	0.00366	0.01525
Average Srednja vrednost	**0.605**		**0.0297**	**0.06437**

Table 2. Quantity of isolated DNA from solid dental tissue from group of premolars
Tabela 2. Količina izolovane DNK iz čvrstog zubnog tkiva iz grupe premolara

Amount of DNA isolated from the dental tissue Količina DNK izolovana iz zubnog tkiva				
	Weight of the tooth (g) Težina zuba (g)	Type of the tooth Vrsta zuba	DNA concentration (ng/µl) Koncentracija DNK (ng/µl)	DNA concentration per gram of the tooth (ng/µl/g) Koncentracija DNK po gramu zuba (ng/µl/g)
1.	0.76	premolar	0.0115	0.015132
2.	1.02	premolar	0.0548	0.053725
3.	1.13	premolar	0.0514	0.045487
4.	1.00	premolar	0.0321	0.0321
5.	0.25	premolar	0.0345	0.138
6.	0.63	premolar	0.0965	0.153174
7.	0.92	premolar	0.0628	0.068260
8.	1.04	premolar	0.0614	0.059038
9.	1.42	premolar	0.0971	0.068380
10.	0.75	premolar	0.0496	0.066133
Average Srednja vrednost	**0.892**		**0.04116**	**0.069942**

Table 3. Quantity of isolated DNA from solid dental tissue from group of molars
Tabela 3. Količina izolovane DNK iz čvrstog zubnog tkiva iz grupe premolara

Amount of DNA isolated from the dental tissue Količina DNK izolovana iz zubnog tkiva				
	Weight of the tooth (g) Težina zuba (g)	Type of the tooth Vrsta zuba	DNA concentration (ng/µl) Koncentracija DNK (ng/µl)	DNA concentration per gram of the tooth (ng/µl/g) Koncentracija DNK po gramu zuba (ng/µl/g)
1.	2.31	molar	0.0169	0.007316
2.	2.64	molar	0.2050	0.077652
3.	1.63	molar	0.0126	0.00773
4.	2.02	molar	0.0976	0.048317
5.	1.55	molar	1.04	1.890909
6.	1.62	molar	0.0219	0.013518
7.	1.84	molar	0.2130	0.115760
8.	0.92	molar	0.0228	0.024782
9.	1.14	molar	0.0926	0.081228
10.	1.38	molar	0.0454	0.032898
Average Srednja vrednost	**1.705**		**0.17678**	**0.230011**

approximately the same concentration of isolated DNA, with slightly more from the group of premolars. This can be explained by the fact that premolars have variations in the crown size and number of roots (one or two roots). Positive correlation was found between the tooth weight and the amount of isolated DNA. The lowest weight had incisors with average of 0.605g, and they gave the least amount of isolated DNA. Then, premolars had average of 0.892g, and they provided more isolated DNA, while molars weighing 1.705 g gave the greatest amount of isolated DNA. Other studies that compared the amount of DNA between different types of teeth showed that teeth with the largest quantity of dental tissue are the best source of DNA [11, 7]. It has also been confirmed that greater amount of DNA can be obtained from teeth with more roots and this is due to the larger root surface that provides greater amount of cement [11].

Teeth with more roots not only have larger root area and higher amount of pulp tissue in which odontoblasts and cementocites are located, but also show more cellular cement, compared to other groups of teeth, which in some cases have little or no cellular cement. Therefore, molars would be teeth of choice of tooth for DNA sampling due to the largest surface area of the root. In the absence of molars, premolars are recommended for DNA isolation, because they have greater amount of cellular cement than anterior teeth, but canine would have larger volume of pulp. Greater amount of cellular cement provides greater number of cementocites, cells that are the main source of DNA.

In the second part of the study, it was shown that the amount of DNA obtained from the pulp was significantly higher than the amount of DNA obtained from hard dental tissues. The amount of DNA obtained from the pulp, by different groups of teeth, indicated that the group of

Table 4. Quantity of isolated DNA from hard dental tissue
Tabela 4. Količina izolovane DNK iz čvrstog zubnog tkiva

Group 2. Amount of DNA isolated from the dental pulp				
	Weight of the tooth (g)	Type of the tooth	DNA concentration (ng/µl)	DNA conc. per gram of the thooth (ng/µl/g of tooth)
1.	0,52	incisor	0,0160	0,030769
2.	0,29	incisor	0,0451	0,155517
3.	1,33	incisor	0,0124	0,009323
4.	1,24	incisor	0,0585	0,047177
5.	0,10	incisor	0,00216	0,0216
Average Srednja vrednost			**Av. 0,0268**	**Av. 0,05287744**
1.	0,76	premolar	0,0115	0,015132
2.	1,02	premolar	0,0548	0,053725
3.	1,13	premolar	0,0514	0,045487
4.	1,00	premolar	0,0321	0,0321
5.	0,25	premolar	0,0345	0,138
Average Srednja vrednost			**Av. 0,0369**	**Av. 0,056888759**
1.	2,31	molar	0,0169	0,007316
2.	2,64	molar	0,2050	0,077652
3.	1,63	molar	0,0126	0,00773
4.	2,02	molar	0,0976	0,048317
5.	0,55	molar	1,04	1,890909
Average Srednja vrednost			**Av. 0,2744**	**Av. 0,406384703**
Average DNA conc. per gram of the tooth 0,17205 ng/µl/g				

Table 5. Quantity of isolated DNA from pulp tissue
Tabela 5. Količina izolovane DNK iz pulpnog tkiva

Group 2. Amount of DNA isolated from the dental pulp				
	Weight of the tooth (g)	Type of the tooth	DNA concentration (ng/µl)	DNA conc. per gram of the thooth (ng/µl/g of tooth)
1.	0,01195	incisor	0,01860	1,55649
2.	0,01049	incisor	0,06300	6,00572
3.	0,00132	incisor	0,02020	2,67905
4.	0,00282	incisor	0,00568	2,014418
5.	0,00725	incisor	0,10200	14,06897
Average Srednja vrednost			**Av. 0,04190**	**Av. 26,32441**
1.	0,00812	premolar	0,02450	3,01724
2.	0,01700	premolar	0,06980	4,10588
3.	0,01658	premolar	0,03360	2,02654
4.	0,00754	premolar	0,07570	57,34848
5.	0,01026	premolar	0,01510	1,47173
Average Srednja vrednost			**Av. 0,04374**	**Av. 67,96987**
1.	0,02808	molar	0,09440	3,506
2.	0,00988	molar	0,31800	0,348
3.	0,01098	molar	0,98200	8,600
4.	0,01856	molar	0,01850	0,863
5.	0,01098	molar	0,03010	0,021
Average Srednja vrednost			**Av. 0,28860**	**Av. 128,72151**
Average DNA conc. from pulps				

molars quantitatively provides the largest amount of DNA. This is due to the fact that pulp tissue of molars has the largest number of present cells that are major source of DNA (Table 5).

The quantity of DNA obtained from pulp complex of three different groups of teeth, showed that molars provided the largest amount of DNA. That was expected, as the volume of pulp chamber in molars is the largest. Group of premolars and incisors provided similar amount of isolated DNA that was six times smaller than from the group of molars. Similarly, the amount of DNA isolated from hard dental tissues was significantly higher in the group of molars than the other two groups (premolars and incisors) that were similar. Comparing the amount of isolated DNA (ng/µl/g) from pulp and hard dental tissue showed that pulp provided 85 times higher amount of DNA.

Dentin / pulp complex constitutes the bulk of the tooth, and unlike the enamel, is highly complex cellular tissue. The pulp is rich vascularized and innervated, and contains connective tissue with various types of cells. These include odontoblasts (cells that produce dentin), fibroblasts, defense cells (macrophages and histocytes), plasma cells, nerve cells, undifferentiated mesenchymal cells [12]. Knowing that about 80 diploid cells are sufficient to provide a minimum amount of DNA required for STR mapping, it can be concluded that the pulp is an extremely valuable source of DNA. Pulp complex is in connection with periodontal tissues (tissues that connect any tooth to the alveolar bone) over the top of the root, and through accessory canals [13].

CONCLUSION

Pulp tissue and cement are clearly the most valuable sources of nuclear DNA from the tooth. Enamel protects dentin and pulp, but has no DNA. Main tissue that should be used for DNA isolation is pulp, but in those cases where it is not present (endodontic treatment), hard dental tissues provide sufficient quantity of DNA for identification procedures. The group of teeth that provides the greatest amount of DNA is molars.

REFERENCES

1. Keiser-Neilsen S. Person Identification by Means of Teeth. Bristol: John Wright & Sons; 1980.
2. Jones DG. Odontology often is final piece to grim puzzle. J Calif Dent Assoc. 1998; 26:650-3. [PMID: 9879231]
3. Manjunath BC, Chandrashekhar BR, Mahesh M, Vatchala Rani RM. DNA profiling and forensic dentistry - A review of the recent concepts and trends. J Forensic Leg Med. 2011; 18:191-7. [DOI: 10.1016/j.jflm.2011.02.005] [PMID: 21663865]
4. Butler JM. Forensic DNA typing: Biology, technology and genetics of STR markers. San Diego: Academic Press; 2005.
5. Buckleton J, Triggs CM, Walsh SJ. Forensic DNA evidence interpretation. Washington, D.C: CRC Press; 2005.
6. O'Rourke DH, Hayes MG, Shawn WC. Ancient DNA studies in physical anthropology. Annu Rev Antrop. 2000; 29:217-42.

7. De Leo D, Turrina S, Marigo M. Effects of individual dental factors on Genomic DNA Analysis. Am J Forensic Med Pathol. 2000; 21:411-5. [PMID: 11111808]
8. Corte-Real AT, Anjos MJ, Addrade M. Genetic identification in endodontic treated tooth root. Forencis Sci Int. Genetics Supplement Series 01/2008; 1:457-8. [DOI: 10.1016/j.fsigss.2007.10.094]
9. Smith BC, Fisher DL, Weedn VW, Warnock GR, Holland MM. A systematic approach to the sampling of dental DNA. J.Forensic Sci. 1993; 38:1194-209. [PMID: 8228888]
10. Malaver PC, Yunis JJ. Different dental tissues as a source of DNA for human identification in forensic cases. Croat Med J. 2003; 44:306–9. [PMID: 12808723]
11. Rubio L, Martinez LJ, Martinez E, Martin de las Hernas S. Study of short and long term storage of teeth and its influence on DNA. J Forensic Sci. 2009; 54:1411-3. [DOI: 10.1111/j.1556-4029.2009.01159.x] [PMID: 19804532]
12. Chiego DJ. Histology of the pulp. In: Avery JK, Steele PF, Avery N (Eds.). Oral Development and Histology, New York: Thieme Medical Publishers; 2002. p. 190–212.
13. Vertucci FJ, Anthony RL. A scanning electron microscopic investigation of accessory foramina in the furcation and pulp chamber floor of molar teeth. Oral Surg Oral Med Oral Pathol. 1986; 62(3):319-26. [PMID: 3462636]

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Forenzička stomatologija – ključ do istine

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KRATAK SADRŽAJ

Uvod Ljudski organizam se može identifikovati kroz testiranje i analizu sekvenci DNK. Najčešći izvor DNK za analizu su krv, meko tkivo, kosa, kosti i zubi. Zubi predstavljaju izbor tkiva za analizu u onim slučajevima u kojima postoji visok stepen degradacije drugih tkiva. Čvrsta zubna struktura pruža zaštitu i očuvanje molekula DNK.
Cilj je bio da se istraži koja grupa zuba i zubnog tkiva (pulpa ili tvrda zubna tkiva) ima najveću količinu DNK.

Materijal i metod Analizirano je 45 ekstrahovanih zuba. U prvom ispitivanju 30 zuba podeljeno je u tri grupe (svaka po 10 zuba): prva grupa su bili sekutići, druga premolari i treća molari. Zubi su mereni pre i nakon postupka izolacije DNK korišćenjem specijalne vage sa preciznošću 0,02–0,000005 ng. Postupak je obuhvatio mlevenje zuba u blenderu i izolaciju DNK pomoću komercijalnih kompleta (izolacija magnetnim česticama). Za drugo ispitivanje analizirano je 15 zuba podeljenih u dve grupe. U prvoj grupi urađena je izolacija molekula DNK iz zubne pulpe, a u drugoj grupi iz tvrdih zubnih tkiva. Kvantifikacija uzoraka napravljena je pomoću Quantifiler® Duo DNA Quantification Kita (Applied Biosystems).

Rezultati Najveća količina DNK dobijena je iz molara (0,0230011 ng/µl/g), dok je najmanja količina DNK dobijena u prvoj grupi (sekutići) i iznosila je 0,06437 ng/µl/g. Takođe, količina DNK izolovane iz tkiva pulpe je bila značajno veća nego iz tvrdih zubnih tkiva (iz pulpnog tkiva kutnjaka dobijena je kvantitativno najveća količina DNK).

Zaključak Glavno tkivo koje treba koristiti za izolaciju DNK iz zuba je pulpa, ali u onim slučajevima kada nije prisutna (endodontski tretman) tvrda tkiva obezbeđuju dovoljnu količina DNK za postupke identifikacije. Grupa zuba koja pruža najveću količinu DNK su kutnjaci.

Ključne reči: DNK izolacija; kutnjaci; pulpodentinski kompleks

UVOD

Forenzička stomatologija predstavlja naučnu disciplinu čiji su interes zub i sve njegove karakteristike, počev od morfologije i analize njegovih strukturnih komponenti, kako bi se dobile informacije za rešavanje krivičnih predmeta ili korišćenje u sudskom (pravnom) sistemu. Forenzička odontologija ili forenzička stomatologija definisana je od strane Keizer-Neilsona 1970. [1] kao „grana forenzičke medicine koja u interesu pravnog sistema analizira i ispituje stomatološke dokaze i na odgovarajući način ocenjuje i predstavlja nalaze". Najčešća uloga forenzičkog zubara je identifikacija preminulih osoba [2]. Identifikacija zuba može biti urađena na dva načina. Prvi je komparativna identifikacija, koja se koristi da bi se utvrdilo da su posmrtni ostaci umrlog lica i osoba koja je predstavljena zubnim podacima dobijenim pre smrti (antemortem) jedna ista osoba. Druga metoda obuhvata slučajeve gde podaci pre smrti (antemortem) ne postoje. U tom slučaju forenzički zubar završava podatke nakon smrti (postmortem) tako što dobija individualne karakteristike pokojnika kako bi ih uporedio sa antemortemskim podacima. Ovaj proces se naziva postmortem zubno profiliranje.

Ljudsko telo se takođe može identifikovati kroz testiranje i analizu sekvenci DNK. Svaka ćelija pojedinca sadrži više kopija DNK. Svako ljudsko biće karakteriše jedinstvena (pojedinačna) DNK sekvenca zbog hipervarijabilnih regiona DNK koji su specifični za tu osobu. Sekvence molekula DNK koje se nazivaju bazni parovi (bp) u svakom pojedincu variraju, sa izuzetkom identičnih blizanaca, i to se naziva polimorfizam sekvence. Dužina polimorfizma kao AATG-AATG (ponovljena dva puta), ili AATG-AATG-AATG (trostruko ponavljanje), pod nazivom Kratko tandem ponavljanje (STR), koristi se u forenzičkoj identifikaciji [3, 4]. Ove ponavljajuće sekvence se zovu DNK otisci prstiju, a postupak njihove identifikacije naziva se tipizacijom, tj. profiliranjem DNK. To je standardna procedura u forenzičkom sistemu za identifikaciju ljudi, krivične slučajeve i utvrđivanje očinstva [5].

Pored izolacije DNK iz kose, kostiju ili osušenog mekog tkiva, zubi daju najbolje iskoristivu DNK za identifikaciju zbog tvrdog zubnog tkiva koji fizički štiti pulpu. Tkivo koje se najčešće koristi za izolaciju DNK je tkivo pulpe i ono predstavlja vezivno tkivo sastavljeno od ćelija (odontoblasta, fibroblasta, endotelnih ćelija, perifernih živaca, nedefinisanih mezenhimalnih ćelija, makrofaga i limfocita). Međutim, u slučajevima gde je endodontski tretman obavljan na zubu i pulpa uklonjena, DNK se može ekstrahovati iz kalcificiranog tkiva, dentina ili dentina i cementa zajedno postupkom mlevenja kako bi se proizveo prah. DNK izolacija je takođe moguća od parodontalnih vlakana i spojenih parodontalnih fragmenata kostiju.

Kvalitet i količina ekstrahovane DNK iz zuba zavisi od nekoliko spoljašnjih i pojedinačnih faktora. Od pojedinačnih faktora posebna pažnja se daje zapremini komore pulpe, koja je različita u različitim grupama zuba. Kao i za druga tkiva, izolacija DNK iz zuba zavisi od spoljnih uticaja kao što su temperatura, vreme smrti, vlažnost, bakterijska i gljivična kontaminacija [6, 7]. Takođe, najbrža degradacija molekula DNK dešava se u prve dve godine, u zavisnosti od spoljašnjih i pojedinačnih faktora [6].

Cilj ovog rada je bio da se ispita koja grupa zuba i zubnog tkiva (pulpa ili tvrda zubna tkiva) ima najveću količinu DNK.

MATERIJAL I METOD

Analizirano je 45 intaktnih zuba (*in vitro* studija) izvađenih na Klinici za oralnu hirurgiju Univerzitetskog stomatološkog kliničkog centra St. Pantelejmon u Skoplju. DNK analize su sprovedene na Institutu za sudsku medicinu, kriminologiju i medicinsku deontologiju. Metod sa magnetnim česticama korišćen je kao čvrsta faza za vezivanje molekula DNK (Prepfiler Kit iz ABI™).

Ova tehnika izolacije DNK (za razliku od starog procesa organske izolacije) zasniva se na ekstrakciji čvrste faze (SPE). Ona koristi kolone napunjene silicijumom ili drugom čvrstom fazom koja selektivno veže (adsorbuje) nukleinske kiseline (DNK ili RNK) u zavisnosti od pH okoline i koncentracije soli u puferu (u našem slučaju čvrsta faza su bile magnetne čestice). Kolone i svi puferi korišćeni za izvođenje ove procedure dolaze u obliku komercijalnih kitova i postupak je izveden u skladu sa uputstvima proizvođača.

Kvantifikacija uzoraka obavljena je pomoću Qunatifiler DNA Quantification Kita (Applied Biosystems) na 7500 sistemu Real Time PCR. Ovaj pristup omogućava kvantifikaciju DNK u inicijalnom uzorku merenjem fluorescentnih signala tokom amplifikacije ciljane sekvence. Primarni cilj PCR kvantifikacije u realnom vremenu je određivanje količine DNK koja može biti „amplificirana" u uzorku. Ulazna količina Quantifiler® Duo Primer Mixa za kvantifikaciju jednog uzorka iznosila je 10,5 µl, a ulazna količina Quantifiler® Duo PCR Reaction Mixa je bila 12,5 µl. Zapremina uzorka dodatog u reakciju za kvantifikaciju je bila 2 µl. Celokupni volumen reakcione mešavine iznosio je 25 µl.

Prva analiza je obuhvatila 30 zuba podeljenih u tri grupe od po 10 zuba: sekutići, premolari i molari. Postupak izolacije DNK iz zuba uključio je mlevenje zuba u blenderu i korišćenje komercijalizovanih kitova (izolacija magnetnim česticama). Ovim metodom zubi koji su prethodno bili podvrgnuti endodontskom tretmanu ne mogu se koristiti za izolaciju DNK [8]. Zubi su prvo ispirani vodom iz česme, a površina dobro očišćena skalpelom i četkom. Onda su zubi držani u ultrazvučnom kupatilu u 1% natrijum-hipohlorita (NaOCl) dva puta po 10 minuta nakon zamene rastvora NaOCl. Ovaj postupak je ponovljen i dva puta sa destilovanom vodom. Procedura čišćenja je trajala oko 40 minuta. Zubi su tada postavljeni u dasku za isparavanje kako bi se osušili preko noći. Osušenim zubima je prvo bila izmerena težina, a potom su samleveni u blenderu za male kosti i zube da bi se dobio zubni prah.

U drugom testu je korišćeno 15 zuba podeljenih u dve grupe. U prvoj grupi je DNK izolovana iz pulpnog tkiva, a u drugoj grupi iz istih zuba je korišćeno čvrsto zubno tkivo za izolaciju DNK. Pulpa je kolektovana pomoću nervnih ekstirpatora nakon izvršenog horizontalnog preseka zuba. Ovaj postupak horizontalnog presecanja zuba u liniji vrata zuba obavljen je kako bi se očuvala okluzalna površina zuba koja je mogla poslužiti za dalju proceduru identifikacije. Ovo razdvajanje je obavljeno korišćenjem dijamantskih borera i mikromotora. Kružnim pokretima diska polako je presecan zub u nivou vrata. Kad je disk bio blizu pulpne komore, zubi su prelomljeni, kako bi se izbeglo pregrevanje i mehaničko oštećenje tkiva pulpe, ali i kontaminacija materijala.

REZULTATI

U prvom testu su analizirane različite grupe zuba, sveže izvađenih sekutića, premolara i molara. To su tri grupe koje se razlikuju po veličini i broju korena, tj. postoje velike morfološke razlike među njima. Rezultati su prikazani u Tabelama 1–3.

U drugom testu su upoređene količine DNK dobijene iz pulpe i tvrdih zubnih tkiva. Rezultati su prikazani u Tabelama 4 i 5.

DISKUSIJA

U forenzičkim slučajevima identifikacije, gde su ljudski ostaci izuzetno oštećeni ili degradirani, zubi i kosti su često jedini izvor DNK [9, 10]. Genetska analiza zuba zavisi od količine prisutne DNK, nivoa degradacije i efikasnosti procesa kolekcije DNK i metoda koji se koriste za izolaciju DNK [11]. Sa trenutnim saznanjima o strukturi zuba i degradacije DNK u zubnom tkivu posle smrti, naš rad nudi optimalan izbor zuba, kako bi se povećala efikasnost ekstrakcije i profiliranja DNK.

Može se videti da je najveća količina DNK dobijena u grupi molara i to 0,230011 ng/µl/g zuba, dok je u grupi sekutića dobijena najmanja količina DNK, tj. 0,06437 ng/µl/g zuba. Koncentracija izolovane DNK iz grupe molara bila je četiri puta veća od grupe premolara i pet puta veća od grupe sekutića. Međusobno upoređivanje grupe premolara i sekutića pokazalo je približno iste vrednosti koncentracije izolovane DNK, sa malo većom količinom u grupi premolara. Ovo se može objasniti činjenicom da premolari pokazuju velike varijacije u veličini krune i broju korena (jedan ili dva korena). Pozitivna korelacija je utvrđena između težine zuba i količine izolovane DNK. Najmanju težinu imali su sekutići, sa prosekom od 0,605 g, i dali su najmanju količinu izolovane DNK. Premolari su imali prosečno 0,892 g i dali su veću količinu DNK, a molari sa prosečnom masom 1,705 g dali su najveću količinu DNK. Druge studije koje su takođe upoređivale količinu DNK između različitih vrsta zuba pokazale su da su zubi sa najvećom količinom zubnog tkiva najbolji izvor DNK [11, 7]. Takođe je potvrđeno da se veća količina DNK može dobiti od zuba sa više korena i to je zbog veće površine korena, koja obezbeđuje veću količinu cementa [11].

Zubi sa više korenova ne samo što imaju veću površinu korena kao i veću količinu pulpnog tkiva u kome se nalaze odontoblasti i cementociti već pokazuju i više ćelijskog cementa u poređenju sa drugim grupama zuba, koji u nekim slučajevima imaju malo ili nimalo ćelijskog cementa. Prema tome, molari bi bili najbolji izbor zuba za uzorkovanje DNK zbog najveće površine korena. U odsustvu molara premolari se preporučuju za izolaciju DNK, jer imaju veću količinu ćelijskog cementa nego prednji zubi, mada očnjaci imaju veći volumen pulpe. Veća količina ćelijskog cementa pruža veći broj cementocita, ćelija koje su glavni izvor DNK.

U drugom delu studije pokazano je da je količina DNK dobijene iz pulpe značajno veća od količine DNK dobijene iz zubnih tvrdih tkiva. Količina DNK dobijene iz pulpe, po različitim grupama zuba, pokazala je da grupa molara kvantitativno obezbeđuje najveću količinu DNK. Pulpno tkivo molara ima zapravo najveći broj ćelija koje su glavni izvor DNK (Tabela 5).

Količina DNK dobijena iz pulpnog kompleksa tri različite grupe zuba pokazala je da molari imaju najveću količinu DNK. To je očekivano, pošto je zapremina pulpne komore kod molara najveća. Grupa premolara i sekutića obezbedila je sličnu količinu izolovane DNK, koja je bila šest puta manja nego u grupi molara. Slično tome, količina DNK izolovane iz tvrdih zubnih tkiva značajno je bila veća u grupi molara nego u druge dve grupe (premolari i sekutići), koje su pokazale sličnu količinu DNK. Upoređivanje količine izolovane DNK (ng/µl/g) iz pulpe i tvrdih zubnih tkiva pokazalo je da pulpa daje 85 puta veću količinu DNK.

Pulpodentinski kompleks predstavlja najveći deo zuba i za razliku od gleđi predstavlja visokokompleksno ćelijsko tkivo. Pulpa je bogato vaskularizovano i inervirano vezivno tkivo koje sadrži

različite vrste ćelija. Tu se nalaze odontoblasti (ćelije koje proizvode dentin), fibroblasti, odbrambene ćelije (makrofagi i histociti), plazma ćelije, nervne ćelije, nediferencirane mezenhimalne ćelije [12]. Znajući da je oko 80 diploidnih ćelija dovoljno da obezbede minimalnu količinu DNK potrebnu za STR mapiranje, može se zaključiti da je pulpa izuzetno vredan izvor DNK. Pulpno tkivo je u vezi sa periodontalnim tkivima (tkiva koja povezuju bilo koji zub sa alveolarnom kosti) preko vrha korena i pomoćnih kanala [13].

ZAKLJUČAK

Pulpno tkivo i cement su najvredniji izvori nuklearne DNK iz zuba. Gleđ štiti dentin i pulpu, ali nema DNK. Glavno tkivo koje treba koristiti za izolaciju DNK je pulpa, ali u onim slučajevima u kojima nije prisutna (endodontski tretman) tvrda zubna tkiva daju dovoljnu količinu DNK za postupke identifikacije. Grupa zuba koja pruža najveću količinu DNK su molari.

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Oral health related habits, knowledge and attittude in children with asthma

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SUMMARY

Introduction The aim of this research was to determine oral hygiene related habits, knowledge and behavior in children with asthma compared to healthy children.

Methodology This study included 136 children, between 6 to 16 years of age, divided into the two groups. The first group included children with asthma (study group - SG), while the second included healthy children (control group - CG). A questionnaire containing three groups of questions related to oral-hygiene and dietery habits as well as oral health related behaviour in children was prepared.

Results The percentage of children from SG that brush teeth several times a day was 60.2% compared to 77.2% of CG children ($p<0.05$). 19.1% of SG group children versus 38.2% of CG children brush their teeth longer than 2 minutes ($p<0.05$). There was no significant difference in the level of knowledge about plaque impact on oral health between the two groups ($p>0.05$). More than half (52.9%) of CG respondents regularly visit dentist, while 50.0% of SG goes regularly ($p<0.01$). 51.5% of CG children visit the pediatric dentist due to preventive reasons, while SG children goes mainly due to rehabilitation of teeth (42.6%). The fear of dental procedures is more pronounced in SG compared to CG children ($p<0.001$).

Conclusion Oral hygiene related habits and oral health behavior were worse in children with asthma, while no difference was found in relation to the knowledge among the observed groups.

Keywords: children; asthma; oral-hygiene habits; knowledge; oral hygiene

INTRODUCTION

Asthma is chronic inflammatory disease of airways, which usually begins in childhood. Once exposed to certain stimuli, airways narrow due to muscle spasm, mucus plugs and swelling of mucous membranes causing limited airflow [1]. It is one of the leading causes of hospitalization in children age.

Asthma treatment aims either to alleviate symptoms or to exhibit antiinflamatory effect. Literature data indicate [2] that some of anti-asthmatic medications affect the composition and the amount of saliva and therefore expose affected persons to increased risk of caries, tooth erosions, increased prevalence of oral mucosa changes, gingivitis, periodontitis, orophageal candidiasis and orofacial irregularities [2]. Therefore, children with asthma require greater attention because they can be considered risky group for developing oral diseases.

Due to oral hygiene importance in childhood many studies have addressed risk factors that can lead to oral diseases. Based on numerous epidemiological studies it has been recognised that parents' oral health related habits as well as their lifestyle, are transmitted to children and thus directly and indirectly affect the occurrence of tooth decay [3].

The fear of dental procedures affects large number of people, regardless of age, gender, physical and mental maturity, level of education, economic status, occupation and in children specifically, parents approach towards dental treatment [4, 5]. "Fear of dentist" is the most common reason for avoiding treatment, causing the absence of adequate dental care, which may lead to deterioration of oral health [5, 6].

Patients that have asthma are risk patients in dental office as dental stress and strong smell can cause asthmatic attack. Also, due to close proximity of the upper respiratory tract, patients with chronic respiratory problems such as asthma represent risk patients in dentistry. Acute asthma attacks may be one of the factors that increase fear of dental visits as well as affect oral health related behavioral model of asthmatic children.

The aim of this study is to investigate oral hygiene related habits, knowledge and behavior of children with asthma compared to healthy children.

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MATERIALS AND METHODS

The research was conducted at the Department of Dentistry, Faculty of Medicine, University of East Sarajevo, according to the recommendations of the Helsinki Declaration and principles of Good Clinical Practice. Parents and children were informed about the purpose of research, and approval for participation was obtained.

This cross sectional study included children between 6 to 16 years of age (10.49 ± 3.28). A total of 136 children were divided into the two groups. Study group (SG) (n = 68) consisted of children with asthma symptoms. The control group (CG) (n = 68) included healthy children. Asthma diagnosis was established by a competent pediatrician or family medicine physician based on the following criteria: existence of typical asthma symptoms, spirometry findings, reaction to asthma medication (reversibility of obstruction), allergy existence, positive family history and absence of other conditions that may give similar symptoms or signs [1].The study was designed in a way that children from the control group corresponded by gender and age to children from the study group.

For the purpose of this study a questionnaire was created and included three groups of questions: about oral hygiene and dietary related habits, oral health knowledge and established behavioral habits related to dental visits.

Obtained data were processed through standard statistical procedures using statistical program SPSS 19.0 for Windows. To test differences between genders and education level of parents, $\chi 2$ test was used. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Asthma was more frequently diagnosed in boys (77.9%) compared to girls (22.1%) ($p<0.05$) (Figure1). High (18.4%) and secondary education (31.6%) were more prevalent among mothers of children in the CG, compared to primary (4.2%) or secondary education (39.7%) of mothers of SG children ($p<0.05$) (Figure 2). The difference was not observed in relation to the level of education of fathers in these two groups ($p>0.05$).

Almost 83% of SG children used only toothbrush and toothpaste for oral hygiene in comparison to the CG children that used mouthwashes (26.5%) as an adjunct to basic hygienic agents (Table 1). 60.2% of SG children brushed their teeth several times a day, while that percentage was 77.2% in the control group. Parents more frequently supervised the CG children while maintaining oral hygiene (28.0%), compared to the parents of the study group 20.6%. Almost 15% of SG group children were not supervised. Both groups consumed cariogenic food and drink, while larger percentage was observed in the study group ($p>0.05$). Good self-observed oral health was noted at 45.6% of children in the study and 75.0% in the control group ($p<0.001$) (Table 2).

52.9% of children in the control group had frequent checkups with dentist while 50.0% of study group children occasionally went to dentist ($p<0.01$) (Table 3). Interestingly 7.4% of SG children have never been to the dentist, compared to 2.9% of children in the control group. CG children (51.5%) would visit dentist for preventive reasons while SG children most frequently (42.6%) visited dentist for dental treatment. Fear of dental interventions was more pronounced in the study group of children compared to control group ($p<0.001$). High percentage of SG children acquired knowledge about oral health importance from parents (55.9), while that prcentage was 61.8% in CG children (Table 3).

DISCUSSION

Literature has shown that childhood asthma is more common in boys, possibly due to physiologically narrower airways and increased muscle tone, which is lost after 10 years of age while girls are more frequently affected by teenage asthma [7]. The results of our research indicated that asthma was more common in boys.

Inadequate knowledge of parents about oral deseases in children with asthma and prevention resulted in an increased incidence of oral diseases. Based on the recent

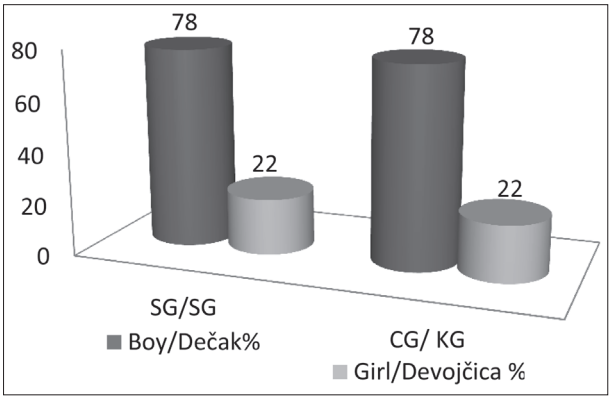

Figure 1. Distribution of children by gender
Grafikon 1. Distribucija dece po polu

SG – Study group, CG – Control group
SG – studijska grupa, KG – kontrolna grupa

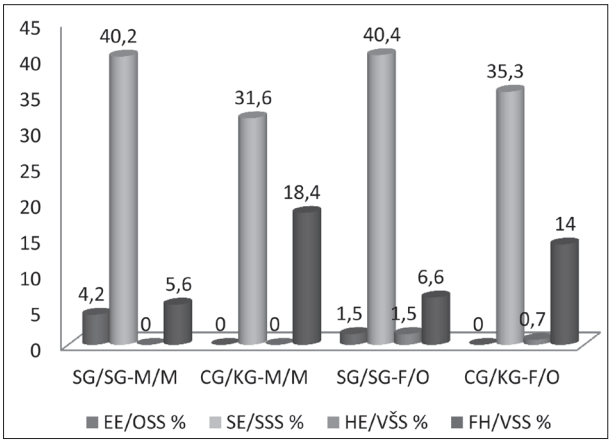

Figure 2. Distribution of parent's education
Grafikon 2. Distribucija školske spreme roditelja

SG – Study group, CG – Control group, M – mother, F – father, EE – elementary education, SE – secondary education, HE – higher education, FE – faculty education

SG – studijska grupa, KG – kontrolna grupa, M – majka, O – otac, OSS – osnovno obrazovanje, SSS – srednjoškolsko obrazovanje, VŠS – visokoškolsko obrazovanje, VSS – fakultetsko obrazovanje

Table 1. Oral hygiene and dietary related habits in the observed groups
Tabela 1. Oralno-higijenske i dijetetske navike kod ispitivanih grupa

Question Pitanje	Answer Odgovor	SG/SG (%) N = 68	CG/KG (%) N = 68	AR/SI (%) N = 136	P
1. To maintain oral hygiene you use? Šta koristiš za održavanje oralne higijene?	Toothbrush and toothpaste Četkicu i pastu za zube	82.4	66.2	74.3	< 0.05
	Toothbrush, toothpaste, floss Četkicu i pastu za zube, konac	7.4	7.4	7.4	
	Toothbrush, toothpaste, mouthwash Četkicu i pastu za zube, tečnost	10.3	26.5	18.4	
2. How often you brush your teeth? Koliko često pereš zube?	Less than once per day Manje od jednom dnevno	6.0	0.0	6.0	< 0.05
	Once a day Jednom dnevno	34.8	22.8	28.8	
	Several times a day Nekoliko puta dnevno	60.2	77.2	68.7	
3. When do you brush your teeth? Kada pereš zube?	In the morning Ujutro	18.6	6.6	12.6	> 0.05
	Before going to bed Pre odlaska u krevet	23.8	13.4	18.6	
	In the morning and in the evening Ujutro i pre odlaska u krevet	51.8	57.8	54.8	
	In the morning, in the afternoon, in the evening Ujutro, popodne i pre odlaska u krevet	5.8	22.2	14.0	
4. How long do you brush your teeth? Koliko dugo pereš zube?	Less than 1 minute Manje od jednog minuta	14.7	8.8	11.8	< 0.05
	1 minute Jedan minut	29.4	17.6	23.5	
	2 minutes Dva minuta	36.8	35.3	36.0	
	More than 2 minutes Duže od dva minuta	19.1	38.2	28.7	
5. My parents ... Moji roditelji me...	Supervise me while I brush my teeth Nadgledaju dok perem zube	20.6	28.0	24.3	> 0.05
	Do not supervise, but advise Ne nadgledaju, ali savetuju	33.8	38.2	36.0	
	Never supervise Nikad ne nadgledaju	14.7	5.8	10.3	
	Only mother supervise Samo majka nadgleda	30.9	27.9	29.4	
6. How often do you consume sweets? Koliko često jedeš slatkiše?	Once a day Jednom dnevno	30.9	32.4	31.6	> 0.05
	Once a week Jednom nedeljno	2.9	4.4	3.7	
	Several times a day Nekoliko puta dnevno	54.4	44.1	49.3	
	Several times a week Nekoliko puta nedeljno	10.3	17.6	14.0	
	I do not eat sweets Ne jedem slatkiše	1.5	1.5	1.5	
7. How often do you drink sweet drinks? Koliko često piješ slatke napitke?	Once a day Jednom dnevno	30.9	26.5	28.7	> 0.05
	Once a week Jednom nedeljno	10.3	10.3	10.3	
	Several times a day Nekoliko puta dnevno	45.6	45.6	45.6	
	Several times a week Nekoliko puta nedeljno	11.8	13.2	12.5	
	I do not drink sweet drinks Ne pijem slatke napitke	1.5	4.4	2.9	

SG – Study group; CG – Control group; AR – All respondents; N – number of respondents; P – statistical significance (Pearson Chi-Square $\chi 2$); % – percentage of the respondents
SG – studijska grupa; KG – kontrolna grupa; SI – svi ispitanici; N – broj ispitanika; P – statistička značajnost (Pearson Chi-Square $\chi 2$); % – procenat ispitanika

Table 2. Oral health related knowledge in the investigated groups
Tabela 2. Stečena znanja u vezi sa oralnim zdravljem kod ispitivanih grupa

Question Pitanje	Answer Odgovor	SG/SG (%) N = 68	CG/KG (%) N = 68	AR/SI (%) N = 136	P
1. What does bleeding of the gums mean? Šta znači kada krvare desni?	Healthy gums Zdrave desni	1.5	2.9	2.2	> 0.05
	Diseased gums Bolesne desni	58.8	55.9	57.4	
	Gums recession Povlačenje desni	0.0	2.9	1.5	
	I do not know Ne znam	39.7	38.2	39.0	
2. How to protect yourself against the occurrence of bleeding gums? Kako se zaštititi od nastanka krvarenja desni?	By the use of toothbrush and pastes Upotrebom četkice i paste	38.2	35.3	36.8	> 0.05
	By the use of soft food Upotrebom mekane hrane	2.9	5.9	4.4	
	By the use of vitamin C Upotrebom vitamina C	4.4	11.8	8.1	
	I do not know Ne znam	54.4	47.1	50.7	
3. What are teeth deposits? Šta su to naslage na zubima?	Soft deposits on the teeth Meke nečistoće na zubima	35.3	23.5	29.4	> 0.05
	Dirty teeth Prljavi zubi	19.1	10.3	14.7	
	Hard deposits on the teeth Tvrde nečistoće na zubima	16.2	22.1	19.1	
	I do not know Ne znam	29.4	44.1	36.8	
4. The presence of teeth deposits leads to... Prisutne naslage na zubima dovode do...	Gingivitis Upale desni	4.4	2.9	3.7	> 0.05
	Dirty teeth Prljavih zuba	16.2	8.8	12.5	
	Tooth decay Karijesa zuba	19.1	17.6	18.4	
	I do not know Ne znam	50.0	45.6	47.8	
	Gingivitis and dental caries Upale desni i zubnog karijesa	10.3	25.0	17.6	
5. How do you rate your oral health? Kako ocenjuješ svoje oralno zdravlje?	Good Dobro	45.6	75.0	60.3	< 0.001
	Satisfactorily Zadovoljavajuće	19.1	14.7	16.9	
	Average Prosečno	20.6	5.9	13.2	
	Neither good nor bad Ni dobro ni loše	11.8	2.9	7.4	
	Bad Loše	2.9	1.5	2.2	

SG – Study group; CG – Control group; AR – All respondents; N – number of respondents; P – statistical significance (Pearson Chi-Square $\chi 2$); % – Percentage of the respondents
SG – studijska grupa; KG – kontrolna grupa; SI – svi ispitanici; N – broj ispitanika; P – statistička značajnost (Pearson Chi-Square $\chi 2$); % – procenat ispitanika

Table 3. Oral health related behavior in observed groups
Tabela 3. Ponašanje prema oralnom zdravlju kod ispitivanih grupa

Question Pitanje	Answer Odgovor	SG/SG (%) N = 68	CG/KG (%) N = 68	AR/SI (%) N = 136	P
1. Did anybody talked to you about the importance of maintaining mouth and teeth healthy? Da li ti je neko pričao o važnosti očuvanja zdravlja usta i zuba?	Parent Roditelj	55.9	61.8	58.8	> 0.05
	Teacher Nastavnik	2.9	0.0	1.5	
	Dentist Stomatolog	16.2	16.2	16.2	
	Family doctor Porodični lekar	1.5	0.0	0.7	
	None Niko	4.4	2.9	3.7	
	Parents and dentists Roditelji i stomatolog	19.1	19.2	19.1	

2. How often do you visit your dentist? Koliko često posećuješ svog stomatologa?	Regularly Redovno	30.9	52.9	41.9	< 0.01
	Periodically Ponekad	50.0	29.4	39.7	
	When I have toothache Kada me boli zub	11.8	14.7	13.2	
	I have never visited the dentist Nikad nisam bio kod stomatologa	7.4	2.9	5.1	
3. The last time you visited the dentist? Zadnji put si posetio/la stomatologa…	I have not been at the dentist until now Nisam bio dosad	7.4	2.9	5.1	< 0.01
	Before 6 months Pre 6 meseci	63.2	58.8	61.0	
	6–12 months 6–12 meseci	13.2	32.4	22.8	
	1–2 years 1–2 godine	14.7	2.9	8.8	
	In last 2–5 years 2–5 godina	1.5	2.9	2.2	
4. How many times have you visited a dentist in last year? Koliko puta si posetio/la stomatologa u protekloj godini?	Not once Nijednom	20.6	10.3	15.4	> 0.05
	Once Jednom	19.1	16.2	17.6	
	Two times Dva puta	11.8	17.6	14.7	
	Three times Tri puta	22.1	14.7	18.4	
	Four and more times Četiri i više puta	26.5	41.2	33.8	
5. Treatment that you had during the last visit to the dentist? Tretman koji si imao/la u toku poslednje posete stomatologu?	I have not been at the dentist until now Nisam bio dosad kod stomatologa	7.4	2.9	5.1	> 0.05
	Examination or fluoridation of teeth Pregled ili fluorisanje zuba	39.7	51.5	45.6	
	Orthodontic treatment Ortodontski tretman	4.4	2.9	3.7	
	Gum treatment Lečenje desni	5.9	1.5	3.7	
	Fillings Postavljanje plombe	23.5	20.6	22.1	
	Tooth extraction Vađenje zuba	19.1	20.6	19.9	
6. If you do not visit a dentist or you are afraid to go to him/her the reason is? Ako ne posećuješ stomatologa ili se bojiš ići kod njega, razlog je?	I'm afraid of dental needles Bojim se stomatološke igle	13.2	7.4	10.3	< 0.001
	I'm uncomfortable due to sound of machines Neprijatno mi je zbog zvuka mašine	0.0	2.9	1.5	
	Since there is no toothache, there is no need to go to the dentist Pošto nema zubobolje, nema potrebe ići stomatologu	13.2	8.8	11.0	
	I'm afraid to even think about a scheduled appointment at the dentist Bojim se čak i razmišljati o zakazanom pregledu kod stomatologa	19.1	2.9	11.0	
	I am not afraid of going to the dentist Ne bojim se odlaska kod stomatologa	54.4	77.9	66.2	

SG – Study group; CG – Control group; AR – All respondents; N – number of respondents; P – statistical significance (Pearson Chi-Square $\chi 2$); % – percentage of the respondents
SG – studijska grupa; KG – kontrolna grupa; SI – svi ispitanici; N – broj ispitanika; P – statistička značajnost (Pearson Chi-Square $\chi 2$); % – procenat ispitanika

study conducted in Croatia, parents can influence their children in terms of oral hygiene maintainance [3]. The study in Iran demonstrated that mothers who took good oral hygiene, transfer their knowledge and attitudes to their children [8]. The level of education and parents incomes influence the incidence of oral diseases [9, 10]. In this study, it was observed that mothers in the study group had lower education than mothers in the control group.

Proper and regular oral hygiene includes daily, often and long enough teeth brushing, as well as application of some additional hygiene methods. Children often do not understand the importance of brushing their teeth [11]. Efficient removal of dental plaque is crucial for the health of teeth and periodontal tissues. Proper habits, attitudes and behavior toward oral health are best to be established in childhood. The results of this research showed that children in the study group irregularly maintain hygiene, do not brush long enough, and mostly use toothbrush and toothpaste only. Unlike our study, research conducted in Sweden showed that there was no difference in oral hygiene maintainince habits between children with and without asthma [12]. Studies of Mazzoleni et al. and Eloot et al. showed that children with asthma had better oral-hygiene habits than healthy control group [13, 14].

Adequate oral hygiene of parents and supervision of children when brushing their teeth are important predictors of good oral health [15]. Supervision of children should be conducted up to 10 years of children's age, until their manual skills are sufficiently developed to properly brush teeth. The results of our study showed that children in the study group were less supervised than children from control group. One of the possible explanations for this phenomenon could be greater devotion of parents to their children's basic illness.

If everyday diet often includs sweet foods, especially between meals, combined with improper oral hygiene maintenance, there is great chance of tooth decay appearance. Analyzing the results of the current study, it was noted that children in the study group consumed more sweets or drunk sweet drinks several times a day. However, frequent consumption of candy and beverage in this group of children did not indicate statistically significant difference in relation to the control group. The results of our research are in accordance with Stensson at al. [12]. Similar eating habits were also reported in children from the experimental and control groups in the study conducted by Mazzoleni at al. in Italy [13]. On the other hand, children with asthma from Belgium consumed less sweets [14]. Higher consumption of non-alcoholic drinks rich in sugars was observed in subjects with asthma from South Australia [16], and Norway [17] compared to their healthy peers. Parents of children with asthma should be adequately educated about possible oral health problems related to food and drinks consumption, especially after inhalation, the importance of fluoride use and maintaining oral hygiene. In some countries, parents of children suffering from asthma in hospital conditions take greater care and attention to improving their children's oral hygiene habits, and thus better control of the disease [14].

Poor oral hygiene and periodontal disease may increase the incidence of lung infections in risky patients. Oral cavity has been considered a potential reservoir for respiratory infections microorganisms as dental plaque may trap respiratory pathogenic microorganisms [18]. Good oral health is important not only to prevent oral diseases but also to maintain good respiratory function. Mechanical removal of soft deposits reduces the number of gram-negative bacteria that also helps keep airways open in children with asthma [19]. Knowledge about dental plaque of all subjects from both groups in our study was poor.

Our study indicated that children with asthma understood the importance of oral health and stated their oral health was not at satisfactory level. However, they visited dentist mainly when they had a specific problem. Studies have confirmed that children who practice dental visits more frequently are better informed about mouth and teeth health [20].

Regular dental checkups should be performed at least twice a year. However, if a person has potentially higher risk of developing oral disease, as seen in asthma [2], examinations should be more frequent. The result of our questionnaire suggested that children with asthma only occasionally went to dentist. Toothache was one of the main reasons for visiting dentist [20, 21, 22], rarely check-up or tooth restoration. These findings are consistant with results of other studies [22, 23].

Wogelius at al. in their research found more frequent presence of dental anxiety in children with asthma [24]. It was more pronounced in younger children [24]. In our study, children with asthma had greater degree of fear even just in planning their visit to the dentist, as well as possible use of dental needles that would cause dental visits delay. Delaying dental visits on the other hand would increase dental fear creating vicious circle [25].

The role of the dentist in advising parents about adequate way to apply preventative measures and preserve oral health has undeniable significance as children acquire first knowledge, attitudes or habits about oral health importance from parents [20].

CONCLUSION

Children with asthma have partially developed oral hygiene habits compared to healthy children. Also, their level of knowledge and behavior toward their own oral health is not adequate. Fear of dental interventions is one limiting factor. For successful dental treatment of children with asthma, good communication skills are important given their previous experience (being in hospital due to asthma, injection therapy, ongoing checkups). It is necessary to emphasize the importance of the first contact with dentist in the earliest age, primarily due to acquisition of positive habits, as well as introduction of preventive measures.

REFERENCES

1. Cover RA, Fleischer DM, Boguniewicz M. Allergic disorders. In: Hay Jr. WW, Levin MJ, Deterding RR, Abzug MJ, Sondheimer JM, editors. Current diagnosis and treatment pediatrics. 21st ed. New York: McGraw Hill; 2012.
2. Thomas MS, Parolia A, Kundabala M, Vikram M. Asthma and oral health: a review. Aust Dent J. 2010; 55(2):128-33. [DOI: 10.1111/j.1834-7819.2010.01226.x] [PMID: 20604752]
3. Beljan M, Puharić Z, Žulec M, Borić D, Neumuller KR. Znanje o oralnom zdravlju i zdravstveno ponašanje roditelja i djece školskog uzrasta. Acta Med Croatica. 2016; 70: 165-71.
4. Vodanović M. Strah od stomatologa. Zdrav život. 2008; 57(7):66-70.
5. Priya SL, Moses J, Rangeeth BN, Sivakumar S. Dental Fear under Rated Yet Significant. Int J Dent Sci Res. 2014; 2(4A):12-4. [DOI: 10.12691/ijdsr-2-4A-4]
6. Carrillo-Diaz M, Crego A, Armfield JM, Romero-Maroto M. Treatment experience, frequency of dental visits, and children's dental fear: a cognitive approach. Eur J Oral Sci. 2012; 120:75-81. [DOI: 10.1111/j.1600-0722.2011.00921.x] [PMID: 22288924]
7. Ivković-Jureković I. Specifičnosti astme dječje dobi. Medicus. 2013; 22(1):43-8.
8. Mohebbi S, Virtanen JI, Murtomaa H, Vehkalahti MM. Mothers as facilitators of oral hygiene in early childhood. Int J Paediatr Dent. 2008; 18:48-55. [DOI: 10.1111/j.1365-263X.2007.00861.x] [PMID: 18086026]
9. Miller E, Lee JY, DeWalt DA, Vann WFJr. Impact of caregiver literacy on children's oral health outcomes. Pediatrics. 2010; 126(1):107-14. [DOI: 10.1542/peds.2009-2887] [PMID: 0547644]
10. Radović I, Davidović L, Krunić J, Stojanović N. Stanje zuba u odnosu na socioekonomske faktore i protetički status kod osoba starije životne dobi u Republici Srpskoj. Stom Glas Srb. 2015; 62(1):14-20. [DOI: 10.1515/sdj-2015-0002]

11. Gill P, Stewart K, Chetcuti D, Chestnutt IG. Children's understanding and motivations for toothbrushing: a qualitative study. Int J Dent Hyg. 2011; 9(1):79-86. [DOI: 10.1111/j.1601-5037.2010.00442.x.] [PMID: 21226855]
12. Stensson M, Wendt L.-K, Koch G, Oldaeus G, Lingström P, Birkhed D. Caries Prevalence, Caries-Related Factors and Plaque pH in Adolescents with Long-Term Asthma. Caries Res. 2010; 44:540–6. [DOI: 10.1159/000321566] [PMID: 21051892]
13. Mazzoleni S, Stellini E, Cavaleri E, Volponi AA, Ferro R, Colombani SF. Dental caries in children with asthma undergoing treatment with short-acting ß2-agonists. Eur J Paediatr Dent. 2008; 9(3):132-8. [PMID: 18844442]
14. Eloot AK, Vanobbergen JN, De Baets F, Martens LC. Oral health and habits in children with asthma related to severity and duration of condition. Eur J Paediatr Dent. 2004; 5(4):210-5. [PMID: 15606319]
15. Lalić M, Aleksić E, Gajić M, Malešević Đ. Oral health related knowledge and health behavior of parents and school children. Med Pregl. 2013; 66(1-2):70-9. [DOI: 10.2298/MPNS1302070L] [PMID: 23534304]
16. Shi Z, Dal Grande E, Taylor AW, Gill TK, Adams R, Wittert GA. Association between soft drink consumption and asthma and chronic obstructive pulmonary disease among adults in Australia. Respirology. 2012; 17(2):363-9. [DOI: 10.1111/j.1440-1843.2011.02115.x.] [PMID: 22142454]
17. Bueso AK, Berntsen S, Mowinckel P, Andersen LF, Lødrup Carlsen KC, Carlsen K-H. Dietary intake in adolescents with asthma – potential for improvement. Pediatr Allergy Immunol. 2011; 22:19-24. [DOI: 10.1111/j.1399-3038.2010.01013.x] [PMID: 20961335]
18. Scannapieco FA, Ho AW. Potential associations between chronic respiratory disease and periodontal disease: Analysis of National Health and Nutrition Examination Survey III. J Periodontol. 2001; 72(1):50–6. [DOI: 10.1902/jop.2001.72.1.50] [PMID: 11210073]
19. Pambudi W, Fabiola I, Indrawati R, Utomo H, Endaryanto A, Harsono A. Changes in bacterial profiles after periodontal treatment associated with respiratory quality of asthmatic children. Paediatrica Indonesiana.2008; 48(6):327-38. [DOI: 10.14238/pi48.6.2008.327-37]
20. Davidović B, Ivanović M, Janković S, Lečić J. Informisanost, stavovi i ponašanje djece prema oralnom zdravlju. Vojnosanit pregl. 2014; 71(10):949-56. [DOI: 10.2298/VSP130714034D] [PMID: 25518275]
21. Al-Omiri MK, Al-Wahadni AM, Saeed KN. Oral Health Attitudes, Knowledge, and Behavior Among School Children in North Jordan. J Dent Educ. 2006; 70(2):179-87. [PMID: 16478932]
22. Obradović M, Dolić O. Caries prevalence and risk factors for its development in urban and rural regions. Stom Glas S. 2008; 55(1):34-42. [DOI: 10.2298/SGS0801034O]
23. Prasad AKP, Shankar S, Sowmya J, Priyaa CV. Oral health Knowledge Attitude Practice of School students of KSR Matriculation School, Thiruchengode. J Ind Aca Dent Spec. 2010; 1(1):5-11.
24. Wogelius P, Poulsen S, Sřrensen HT. Asthma, ear problems, and dental anxiety among 6- to 8-yr-olds in Denmark: a population-based cross-sectional study. Eur J Oral Sci. 2003; 111(6):472-6. [PMID: 14632682]
25. Armfield JM. What goes around comes around: revisiting the hypothesized vicious cycle of dental fear and avoidance. Community Dent Oral Epidemiol. 2013; 41(3):279-87. [DOI: 10.1111/cdoe.12005] [PMID: 23004917]

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Oralnohigijenske navike kod dece obolele od astme

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KRATAK SADRŽAJ

Uvod Cilj ovog istraživanja je bio da se na osnovu upitnika ispita poznavanje oralnohigijenskih navika, znanja i ponašanja vezanog za oralno zdravlje dece sa astmom.

Metode rada U istraživanje je uključeno 136 dece uzrasta od 6 do 16 godina, podeljenih u dve grupe. Prvu – studijsku grupu (SG) – činila su deca sa astmom, dok su u drugu – kontrolnu grupu (KG) – bila uključena zdrava deca. Za potrebe istraživanja kreiran je anketni upitnik koji je sadržavao tri grupe pitanja: oralnohigijenske i dijetetske navike, znanje, te ponašanje dece prema oralnom zdravlju.

Rezultati Najveći procenat dece studijske grupe (60,2%) zube četka više puta u toku dana u odnosu na 77,2% dece kontrolne grupe ($p < 0,05$). Duže od dva minuta četka 19,1% dece studijske, odnosno 38,2% kontrolne grupe ($p < 0,05$). Nije bilo značajne razlike u nivou znanja o značaju i uticaju plaka na oralno zdravlje između grupa ($p > 0,05$). Veća polovina (52,9%) kontrolne grupe redovno posećuje stomatologa, dok 50% studijske to čini povremeno ($p < 0,001$). Kao razlog zadnje posete stomatologu, preventivnu kontrolu je navelo 51,5% dece kontrolne grupe, dok su deca studijske grupe dolazila uglavnom zbog sanacije zuba (42,6%). Strah od stomatoloških intervencija bio je izražajniji kod dece studijske u odnosu na kontrolnu grupu ($p < 0,001$).

Zaključak Oralnohigijenske navike i ponašanje u vezi sa oralnim zdravljem bili su lošiji kod dece sa astmom, dok nije bilo razlike u nivou stečenih znanja kada su ispitivane grupe u pitanju.

Ključne reči: deca; astma; oralnohigijenske navike; znanja; oralna higijena

UVOD

Astma predstavlja hroničnu zapaljensku bolest disajnih puteva, koja uglavnom započinje već u detinjstvu. Nakon što se izlože određenim podražajima, disajni putevi se zbog grča muskulature, čepova sluzi i otoka sluznice sužavaju, a protok vazduha postaje ograničen. Ovo je jedan od vodećih razloga hospitalizacije u dečjem uzrastu [1].

Terapijska procedura astme ima za cilj ili da ublaži simptome ili da deluje protivupalno. Literaturni navodi ukazuju da neki od antiasmatskih lekova utiču na promenu sastava i količine pljuvačke, tako da su obolele osobe izložene većem riziku od karijesa, erozijama na zubima, povećanoj prevalenci promena na oralnoj sluznici, gingivitisima, parodontopatijama, orofagealnoj kandidijazi, kao i orofacijalnim nepravilnostima [2]. Zbog svega navedenog, deca kojima je dijagnostikovana astma zahtevaju veću pažnju, jer mogu biti rizična grupa za nastanak oralnih oboljenja.

Zbog važnosti oralne higijene u dečjem uzrastu, mnoga istraživanja su rađena na temu prepoznavanja faktora rizika koji mogu dovesti do nastanka oralnih oboljenja. Literaturni navodi ukazuju kako se navike roditelja u vezi sa oralnim zdravljem prenose na decu i tako direktno ili indirektno utiču na pojavu karijesa kod dece [3].

Strah od stomatoloških intervencija prisutan je kod velikog broja ljudi nezavisno od starosti, pola, stepena fizičke i mentalne zrelosti, stepena obrazovanja, ekonomskog statusa, zanimanja, a kad je reč o deci, osim pomenutog, i od odnosa roditelja prema stomatološkom tretmanu [4, 5]. Kod dece prisustvo „straha od stomatologa" predstavlja najčešći razlog za izbegavanje tretmana, zbog čega izostaje odgovarajuća stomatološka zaštita, što može dovesti do pogoršanja oralnog zdravlja [5, 6].

Imajući u vidu da neke stomatološke intervencije mogu biti stresne, da jaki mirisi u stomatološkoj ambulanti mogu provocirati asmatski napad, kao i zbog blizine gornjih disajnih puteva, pacijenti sa hroničnim respiratornim problemima, kakva je astma, predstavljaju pacijente sa rizikom. Akutni asmatični napad može da predstavlja jedan od faktora koji povećavaju strah od odlaska stomatologu, kao i da utiče na model ponašanja dece sa astmom prema oralnom zdravlju.

Cilj ovog istraživanja je bio da se na osnovu upitnika ispita poznavanje oralnohigijenskih navika, stečenih znanja i ponašanja vezanog za oralno zdravlje dece sa astmom.

METODE RADA

Istraživanje je obavljeno na odseku Stomatologija, Medicinskog fakulteta Univerziteta u Istočnom Sarajevu, a prema preporukama Helsinške deklaracije i principima Dobre kliničke prakse. Roditelji i deca su informisana o svrsi istraživanja i dobijena je pismena saglasnost roditelja za učešće u istom.

Istraživanje predstavlja studiju preseka koja je obuhvatila decu starosti od 6 do 16 godina ($10,49 \pm 3,28$). Celokupni uzorak od 136 dece podeljen je u dve grupe. Studijsku grupu (SG) ($n = 68$) činila su deca kojima je dijagnostikovana astma. Kontrolnu grupu (KG) ($n = 68$) predstavljala su zdrava deca, tj. ona kod kojih nisu uočeni znaci bilo kog hroničnog oboljenja. Dijagnoza astme je postavljena od strane nadležnog pedijatra ili lekara porodične medicine, na osnovu sledećih kriterijuma: postojanja tipičnih simptoma astme, nalaza spirometrije, reakcije na lekove za astmu (reverzibilnost opstrukcije), postojanja alergije, pozitivne porodične anamneze, nepostojanja drugih stanja koja mogu da daju slične simptome ili znake [1]. Studija je dizajnirana tako da deca iz kontrolne grupe prema polu i uzrastu odgovaraju deci iz studijske grupe.

Za potrebe ovog istraživanja kreiran je anketni upitnik, koji je sadržavao tri grupe pitanja: o oralnohigijenskim i dijetetskim navikama, stečenim znanjima koja deca imaju u odnosu na oralno zdravlje te formiranim navikama ponašanja kad je reč o posetama stomatologu.

Dobijeni podaci u toku ovog ispitivanja su numerički obrađeni standardnim statističkim procedurama u statističkom

programu SPSS 19,0 za Windows. Za testiranje razlike između polova, stepena stručne spreme roditelja, anketnih pitanja po grupama posmatranja upotrebljen je χ^2 test. Vrednosti $p < 0,05$ smatrane su statistički značajnim.

REZULTATI

Astma je češće dijagnostikovana kod dečaka (77,9%) u odnosu na devojčice (22,1%) ($p < 0,05$) (Grafikon 1). Stečena visoka (18,4%) i srednja stručna sprema (31,6%) bila je zastupljenija kod majki dece iz kontrolne grupe, u odnosu na stečeno osnovno (4,2%) ili srednje obrazovanje (39,7%) majki dece studijske grupe . Ali značajna razlika nije uočena (Grafikon 2).

Skoro 83% dece studijske grupe za održavanje oralne higijene koristi samo četkicu i pastu za zube u odnosu na decu kontrolne grupe, koja uz osnovna sredstva za higijenu upotrebljavaju još tečnosti za ispiranje usta (26,5%) (Tabela 1). Deca studijske grupe u najvećem procentu (60,2%) zube peru više puta u toku dana, dok deca kontrolne grupe tu naviku upražnjavaju u procentu od 77,2. Roditelji češće kontrolišu decu kontrolne grupe dok održavaju oralnu higijenu (28%), u odnosu na roditelje dece studijske grupe (20,6%). Oko 15% dece studijske grupe roditelji ne pomažu niti nadziru pranje zuba. I jedna i druga grupa vole da konzumiraju slatkiše i zaslađene napitke, s tim da to čine u većem procentu deca studijske grupe ($p > 0,05$) (Tabela 1).

Dobro oralno zdravlje prema sopstvenoj proceni navelo je 45,6% deca studijske, odnosno 75% dece kontrolne grupe ($p < 0,001$) (Tabela 2).

U najvećem procentu, na redovne preglede kod stomatologa odlazi 52,9% dece kontrolne grupe, dok 50% dece studijske grupe povremeno odlazi stomatologu ($p < 0,01$) (Tabela 3). U studijskoj grupi 7,4% dece dosad nikad nisu bili kod stomatologa, u odnosu na 2,9% dece iz kontrolne grupe. Deca iz kontrolne grupe (51,5%) kao glavni razlog posete stomatologu navode preventivne razloge, dok su se deca studijske grupe u najvećem broju slučajeva (42,6%) obratila zbog sanacije zuba. Strah od stomatoloških intervencija bio je izražajniji kod dece studijske grupe u odnosu na kontrolnu ($p < 0,001$). Saznanja o važnosti oralnog zdravlja deca studijske grupe u najvećem procentu dobijala su od roditelja (55,9), dok su te vrednosti iznosile 61,8% kod dece kontrolne grupe (Tabela 3).

DISKUSIJA

Literaturni navodi pokazuju da je astma u detinjstvu češća kod dečaka, verovatno zbog fiziološki užih disajnih puteva te povećanog mišićnog tonusa, koji se gubi nakon desete godine života, dok u pubertetu od astme češće obolevaju devojčice [7]. Rezultati našeg istraživanja ukazuju da je astma bila zastupljenija kod dečaka.

Nedovoljna upućenost roditelja o samom oboljenju i načinima prevencije rezultiraju povećanom incidencom pojave oralnih oboljenja kod dece sa astmom. Na osnovu nedavno sprovedene studije u Hrvatskoj utvrđeno je da roditelji mogu uticati na svoje dete u pogledu održavanja oralne higijene [3]. Studija u Iranu je ukazala da majke koje brinu o ličnoj oralnoj higijeni prenose svoje znanje i stavove na decu [8]. Nivo obrazovanja i prihodi roditelja takođe utiču na incidencu nastanka oralnih oboljenja [9, 10]. U ovom istraživanju je uočeno da su majke dece studijske grupe imale niže obrazovanje u odnosu na majke dece iz kontrolne grupe.

Pravilna i redovna oralna higijena podrazumeva svakodnevno, često i dovoljno dugo četkanje zuba, kao i primenu nekih od dodatnih sredstava za higijenu. Deca često nemaju jasnu predstavu o tome zašto je neophodno da peru zube [11]. Efikasno uklanjanje dentalnog plaka presudno je za zdravlje zuba i parodontalnih tkiva. Pravilne navike, stavovi i ponašanje prema oralnom zdravlju najbolje se uspostavljaju u detinjstvu. Rezultati ovog istraživanja su pokazali da deca iz studijske grupe neredovno održavaju higijenu, to čine nedovoljno dugo, i uglavnom upotrebljavaju samo četkicu i pastu za zube. Za razliku od naše studije, jedno istraživanje sprovedeno u Švedskoj je ukazalo na nepostojanje razlika u navikama u održavanju oralne higijene između dece sa astmom i dece bez astme [12]. Studije Mazzoleni i sar., kao i Eloot i sar. pokazale su da su deca sa astmom imala bolje oralnohigijenske navike u odnosu na zdravu kontrolnu grupu [13, 14].

Adekvatna oralna higijena roditelja i navika da kontrolišu dete pri pranju zuba su značajni prediktori dobrog oralnog zdravlja deteta [15]. Kontrola dece u toku održavanja oralne higijene od strane roditelja trebalo bi da postoji do desete godine života, odnosno do momenta kad su njihove manuelne spretnosti dovoljno razvijene da mogu samostalno korektno izvršiti ovu dosta komplikovanu veštinu „pravilnog četkanja zuba". Rezultati ovog istraživanja pokazuju da su deca studijske grupe u većem procentu bila bez roditeljskog nadzora kad je reč o kontroli oralne higijene. Kao jedno od mogućih objašnjenja za ovu pojavu mogla bi biti veća posvećenost roditelja osnovnom oboljenju svog deteta.

Ukoliko se u lancu svakodnevne ishrane nađe često unošenje zaslađene hrane, posebno između obroka, udruženo sa nepravilnim održavanjem oralne higijene, velika je šansa da će se kod osobe pojaviti karijes. Analizom rezultata ovog istraživanja primećeno je da deca studijske grupe češće konzumiraju slatkiše ili piju slatke napitke, što čine i po nekoliko puta u toku dana. Međutim, učestala konzumacija slatkiša i napitaka kod ove grupe dece nije ukazala na statistički značajnu razliku u pojavi karijesa u odnosu na kontrolu. Rezultati našeg istraživanja u skladu su sa istraživanjem Stenssona i sar. [12]. Slične prehrambene navike imala su deca iz eksperimentalne i kontrolne grupe studije sprovedene od strane Mazzoleni i sar. u Italiji [13]. S druge strane, vršnjaci sa astmom iz Belgije konzumirali su u manjem procentu slatkiše [14]. Veća potrošnja bezalkoholnih pića bogatih šećerima uočena je kod ispitanika sa astmom iz Južne Australije [16], ali i kod dečaka iz Norveške koji boluju od astme [17] u odnosu na zdrave vršnjake.

Deca sa astmom, kao i njihovi roditelji, trebalo bi da budu adekvatno upoznati sa mogućim oralnozdravstvenim tegobama vezanim za količinu i učestalost konzumacije pića i hrane (posebno nakon inhalacije), upotrebi fluorida i redovnoj higijeni. U pojedinim zemljama roditelji dece koja se leče od astme u bolničkim uslovima pridaju veću brigu i pažnju svojoj deci u cilju poboljšanja oralnohigijenskih navika, pa samim tim i bolje kontrole bolesti [14].

Loša oralna higijena i parodontopatije mogu uticati na učestalost plućnih infekcija kod rizičnih pacijenata. Usna duplja se smatra potencijalnim rezervoarom za mikroorganizme respiratornih infekcija, jer dentalni plak može služiti kao skladište

respiratornih patogenih mikroorganizama [18]. Dobro oralno zdravlje je važno ne samo da bi se sprečila oboljenja usta već i da bi se održao dobar kvalitet respiratorne funkcije. Mehaničkim uklanjanjem mekih naslaga smanjuje se broj gram-negativnih bacila, a time se poboljšava kvalitet disajnih puteva dece sa astmom [19]. Znanje ispitanika iz obe ispitivane grupe ove studije, procenjivano anketnim upitnikom, bilo je na niskom nivou kad je reč o informacijama o dentalnom plaku.

Ovo ispitivanje ukazalo je na činjenicu da kod dece sa astmom postoji razvijena svest o sopstvenom oralnom zdravlju, i izjasnili su se da njihovo oralno zdravlje nije na zadovoljavajućem nivou. Međutim, iako su toga svesni, stomatologa posećuju uglavnom kad imaju konkretan problem. Istraživanja potvrđuju da su deca koja češće posećuju stomatologa bolje informisana o zdravlju usta i zuba [20].

Redovne stomatološke preglede trebalo bi organizovati najmanje dva puta godišnje. Međutim, ako je osoba u potencijalno većem riziku za nastanak oralnih oboljenja, kao što je to uočeno kod dece obolele od astme [2], preglede bi trebalo organizovati češće. Rezultat ovog anketnog upitnika govori u prilog tome da deca sa astmom povremeno odlaze kod stomatologa. Zubobolja je jedan od glavnih razloga posete dece stomatologu [20, 21, 22], nešto ređe su to kontrolni pregledi ili sanacije zuba [22, 23], a deca sa astmom se u znatnoj meri rukovode takvom praksom.

Wogelius i sar. su u svom istraživanju ukazali na češće prisustvo dentalne anksioznosti kod dece koja boluju od astme [24]. Ona je bila više izražena kod mlađe dece [24]. Na osnovu analize ovog istraživanja deca sa astmom su pokazala veći stepen straha i pri samoj pomisli o zakazanom odlasku kod stomatologa, odnosno mogućoj upotrebi stomatološke igle. Ova vrsta straha je često značajan ograničavajući faktor koji vodi odgađanju poseta stomatologu. Na taj način se samo pojačava strah od stomatoloških intervencija i stvara začarani krug [25].

Uloga stomatologa u savetovanju dece i roditelja u pronalaženju adekvatnog načina za primenu preventivnih mera u očuvanju oralnog zdravlja su od neprocenjivog značaja [20]. Ova studija je ukazala da većina dece prva saznanja, stavove ili navike o važnosti oralnog zdravlja stiču od roditelja, a ne od stomatologa.

ZAKLJUČAK

Deca sa astmom imaju delimično razvijene oralnohigijenske navike u odnosu na zdravu decu, ali nivo saznanja i ponašanje prema sopstvenom oralnom zdravlju nisu odgovarajući. Strah od stomatoloških intervencija je takođe jedan od ograničavajućih faktora. Za uspešan stomatološki tretman dece sa astmom važna je, pre svega, dobra komunikacija sa stomatologom, koji treba da svojim pozitivnim stavom olakša komunikaciju i ukaže na značaj održavanja oralne higijene, odnosno predloži uvođenje preventivnih mera kod dece sa astmom.

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CASE REPORT
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Combined surgical and orthodontic treatment of impacted second lower premolar – Case report

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SUMMARY

Impacted tooth is a tooth that has failed to reach occlusal plane, with 2/3 of completed root growth. There are various reasons for teeth impaction, however, lack of space for its emergence is considered to be the main reason. The most commonly impacted teeth are lower wisdom teeth, then upper wisdom teeth, upper canines, and less frequently lower premolars. There are only few studies that have addressed the issue of impacted lower premolars, their etiology, prevalence and treatment. The patient (22 years old) visited orthodontst for the lower jaw aesthetic teeth corrections, due to crowding. Ortopantomography analysis revealed impacted lower right second premolar that caused resorption of mesial root of the first molar. The treatment plan included tooth #46 extraction and placing orthodontic brackets on the impacted second premolar. The next step was extrusion of the tooth #45, its introduction to occlusal plane and orthodontic treatment resumption. Orthodontic treatment of impacted teeth with fixed orthodontic appliance provides excellent therapeutic results. The treatment success depends primarily on adequate planning, cooperation and joint work of oral surgery and orthodontic specialists.

Keywords: impacted lower premolar; orthodontic extrusion; tooth extraction; root resorption

INTRODUCTION

Impacted lower premolars are not so rare in everyday dental practice. Lower premolars are most frequently impacted teeth, after lower and upper third molars and upper canines [1]. Mandibular premolars erupt after first lower molars and canines, so the lack of space for the eruption of both premolars may lead to impaction of one of them, usually the second premolars [2]. Some of the reasons for their impaction are: lack of space, ectopic position of the tooth bud, presence of obstacles (primary tooth, tumor, scar tissue) on the way of eruption, presence of supernumerary teeth or odontomas. Some of the systemic and genetic diseases such as ccd dysplasia, osteoporosis, Down syndrome, hypothyroidism and hypopituitarism may affect premolars eruption as well [3-5].

Treatment of impacted mandibular premolars depends on tooth position, depth of impaction, relationship with surrounding teeth, as well as planned orthodontic treatment. Treatment includes teamwork: orthodontist who makes treatment plan and oral surgeon who performs surgery to allow access to the impacted tooth. The treatment procedure involves surgical release of the impacted premolar`s crown, bonding orthodontic bracket and further fixed orthodontic treatment. One of the conditions for successful therapy is that the angle of the impacted premolars does not exceed 45° [6].

This case report presents surgical release of impacted lower right second premolar with subsequent orthodontic treatment.

CASE REPORT

The patient (22 years old) presented for an orthodontic examination in order to address the problem of irregular position of the tooth (lack of space) in the lower jaw (Figure 1). After ortopantomography analysis it was observed that lower right second premolar is impacted with suspected resrption of mesial roots of the the first molar. Also, the first lower left premolar was noticed to be missing as well as unerupted wisdom tooth in the fourth quadrant (Figure 2). The patient was healthy and did not have any previous tooth extraction or orthodontic intervention in dental history. Also, he did not report pain or discomfort in the orofacial region.

Orthodontist sent patient to oral surgeon for consultation. Mutual treatment plan was done which included combined surgical-orthodontic treatment. It included extraction of the first lower right molar (#46) and then orthodontic extrusion of the lower right second premolar (#45). First molar was suggested to extraction primarily due to the resorption of mesial root but also making enough room for premolar.

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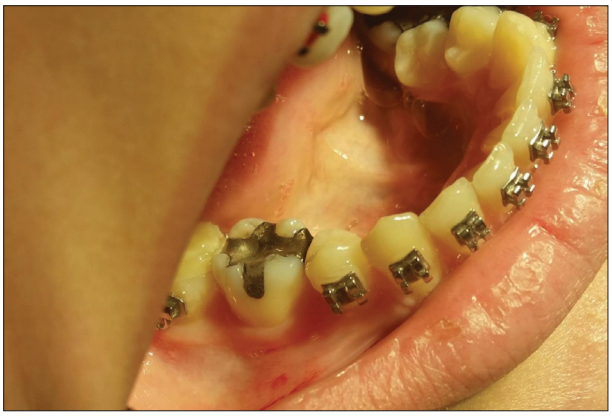

Figure 1. Overview of the situation in the mouth
Slika 1. Prikaz stanja u ustima

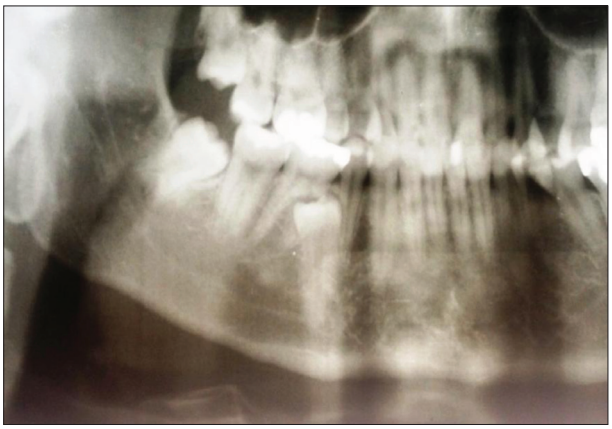

Figure 2. Ortopantomography
Slika 2. Ortopantomogram

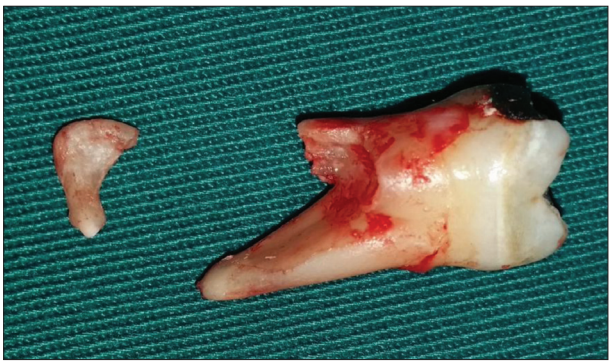

Figure 3. Extracted tooth 46
Slika 3. Ekstrahovan zub 46

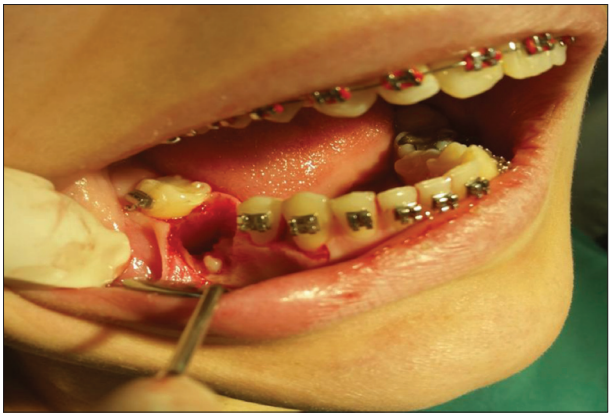

Figure 4. Elevated flap after tooth extraction 46 and tooth crown 45 visible
Slika 4. Podignut režanj nakon vađenja zuba 46 i eksponirana krunica zuba 45

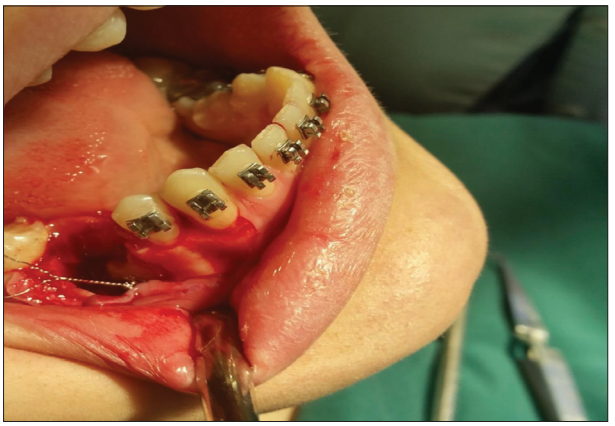

Figure 5. Orthodontic brackets placed on the tooth 45
Slika 5. Ortodontska bravica postavljena na zub 45

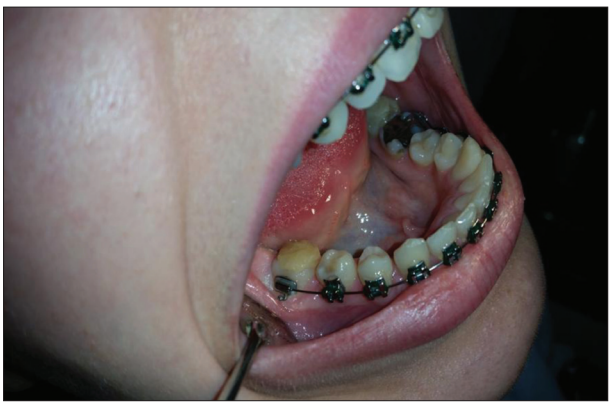

Figure 6. The tooth 45 reached occlusal plane in the dental arch
Slika 6. Zub 45 u zubnom nizu, dostignuta okluzalna ravan

Oral surgery was performed under local anesthesia after the placement of fixed orthodontic appliance. A corner gingival flap of full thickness was carefully lifted between canine and second molar, with taking care of the mental nerve. The tooth #46 was extracted carefully with forceps when midroot resorption of the mesial root caused by emergence of the impacted premolar #45 was observed. The remaining apical part of the root was extracted as well (Figure 3). The entire crown of the impacted second premolar was exposed (Figure 4). The bone around the crown of #45 was removed with carbide burs under constant cooling with sterile saline. This allowed orthodontic bracket placement on the tooth (Figure 5). Individual sutures were placed for 7 days. The patient was prescribed antibiotics (Dovicin 100 mg, 7 days), Chymoral Forte (5 days) for quicker resorption of edema and hematoma and pain killers as needed.

Further orthodontic treatment was focused on the extrusion of the impacted premolar over the next 6 months, its positioning in the dental arch and reaching the occlusal plane (Figure 6). At the same time the lack of space was corrected as well. Orthodontic therapy will be fully completed after wisdom tooth emergence in the fourth quadrant that will fully stabilize occlusion in lateral region.

DISCUSSION

Literature does not provide sufficient data related to the impacted lower premolars, regardless the fact that frequency of these tooth impaction is quite high [7]. The treatment of these teeth is multidisciplinary and includes cooperation of orthodontics with oral and maxillofacial surgeons, pediatric dentists and sometimes endodontists [8]. Treatment plan depends on several factors, primarily on the ammount of space available to initiate the emergance of impacted tooth, depth of impaction, degree of root formation, need for first molar extraction, duration of the therapy and presence of keratinized gingiva. In addition, treatment plan is significantly influenced by the patient`s state of general health, other teeth and oral hygiene, as well as function and occlusion [9,10]. Andreasen recommended surgical exposure of impacted premolar to be limited to cases where the angulation of the tooth is not greater than 45° in both jaws. However, in practice, we can find cases of surgical and orthodontic treatment of horizontally impacted mandibular premolars that were successfully placed in their dental line position [8].

In cases where OPT and clinical examination show lack of space for impacted mandibular premolars, regular checkups are needed, extraction of the primary teeth as well as monitoring of the permanent premolars position. If necessary, surgical release of the tooth crown, with or without orthodontic traction or repositioning (autotransplantation) may be performed. However, if impossible to align impacted mandibular premolar in dental arch then surgical removal of the tooth should be done [11].

To align impacted mandibular premolar, orthodontic treatment should be divided into the three phases. The first phase starts 2 to 5 months after surgical exposure of a tooth. The second stage starts when tooth is tracted to its position in dental arch and lasts 12 to 18 months. The third stage is the end of orthodontic treatment when the tooth is in its place in the arch, having in mind that additional 10 to 18 months are required for completion of orthodontic treatment [12].

One of the possible complications of impacted mandibular premolars is the occurrence of developmental cyst of odontogenic origin [3]. These cysts are common in impacted, retained or developing teeth. As they give none or minor symptomatology they are usually diagnosed accidentally during routine examination or radiography. These cystic lesions often reach large dimensions that may lead to mobility and migration of surrounding teeth or resorption of their roots. Therefore, complete removal of cystic lesions (cystectomy) with impacted tooth extraction are necessary [13]. Sometimes, in order to keep the tooth, marsupialization, that has aim to decompress and fenestrate cystic wall is performed with release of impacted tooth and continuation of the orthodontic treatment [14].

There is an increasing number of young patients with impacted permanent teeth problem (not just wisdom teeth) and successful correction of existing orthodontic anomaly is often complex and lengthy process. Careful and thorough treatment planning, as well as good cooperation of oral surgeon and orthodontists, is crucial to achieving treatment success. Teamwork, regular checkups and good patient cooperation eventually lead to excellent results.

REFERENCES

1. Andreasen JO. The impacted premolar. In: Andreasen JO, Petersen JK, Laskin DM, editors. Textbook and color atlas of tooth impactions; diagnosis, treatment and prevention. Copenhagen: Munksgaard; 1997. p. 177-95.
2. Peterson LJ. Principles of management of impacted teeth. Contemporary Oral and Maxillofacial Surgery. 4th ed. Philadelphia, PA: Mosby; 2003. p. 185.
3. Mishra R, Tripathi AM, Rathore M. Dentigerous Cyst associated with Horizontally Impacted Mandibular Second Premolar. Int J Clin Pediatr Dent. 2014 Jan-Apr; 7(1):
4. 54-7. [DOI: 10.5005/jp-journals-10005-1235]
5. Mariano RC, Mariano Lde C, de Melo WM. Deep impacted mandibular second molar: a case report. Quintessence Int. 2006; 37:773-6. [PMID: 17078275]
6. McDonald RE, Avery DR. Eruption of the teeth: local, systemic and congenital factors that influence the process. In: Dentistry for the child and adolescent. 6th ed. Mosby; 1994. p. 186-213.
7. Collett AR. Conservative management of lower second premolar impaction. Aust Dent J. 2000; 45:279-81. [DOI: 10.1111/j.1834-7819.2000.tb00264.x] [PMID: 11225531]
8. Topkara A, Sari Z. Impacted teeth in a turkish orthodontic patient population: prevalence, distribution and relationship with dental arch characteristics. Eur J Paediatr Dent. 2012 Dec; 13(4):311-6. [PMID: 23270290]
9. Aizenbud D, Levin L, Lin S, Michtei EE. A multidisciplinary approach to the treatment of a horizontally impacted mandibular second premolar: 10-year follow-up. Orthodontics (Chic.). 2011 Spring; 12(1):48-59. [PMID: 21789290]
10. Kaczor-Urbanowicz K, Zadurska M, Czochrowska E. Impacted Teeth: An Interdisciplinary Perspective. Adv Clin Exp Med. 2016; 25(3):575-85. [DOI: 10.17219/acem/37451] [PMID: 27629748]
11. Proffit WR, Field HW. Contemporary Orthodontics. 3rd ed. St. Louis, Mo, USA: Mosby; 2000.
12. Şimşek-Kaya G, Melih-Ömezli M, Yapıcı G, Dayı E, Ertaş Ü. Prevalence of impacted premolars in a Turkish population and considerations for surgical treatment. Med Oral Patol Oral Cir Bucal. 2011 Sep 1;16(6):e781-6. [DOI: 10.4317/medoral.17027] [PMID: 21196868]
13. Wasserstein A, Shalish M. Adequacy of mandibular premolar position despite early loss of its deciduous molar. ASDC J Dent Child. 2002; 69:254-8, 233-4. [PMID: 12613307]
14. An S, Manjunatha B, Astekar M, C.S. Dentigerous cyst associated with ectopically impacted maxillary second premolar. J Exp Ther Oncol. 2016 Nov; 11(4):309-13. [PMID: 27849342]
15. Qian WT, Ma ZG, Xie QY, Cai XY, Zhang Y, Yang C. Marsupialization facilitates eruption of dentigerous cyst-associated mandibular premolars in preadolescent patients. J Oral Maxillofac Surg. 2013 Nov; 71(11):1825-32. [DOI: 10.1016/j.joms.2013.06.223] [PMID: 23973048]

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Hirurško-ortodontska terapija impaktiranog drugog donjeg premolara – prikaz slučaja

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KRATAK SADRŽAJ

Impaktirani zub je onaj koji nije uspeo da dosegne okluzalnu ravan, sa završenim rastom 2/3 korena. Različiti su razlozi impakcije zuba, ali glavnim razlogom se smatra nedostatak prostora za njegovo nicanje. Najčešće impaktirani zubi su donji umnjaci, zatim gornji umnjaci, gornji očnjaci i, nešto ređe, donji premolari. Postoji jako malo studija koje su se bavile problemom impaktiranih donjih premolara, njihovom etiologijom, prevalencom i terapijom. Pacijentkinja (22) javila se na pregled kod ortodonta zbog korekcije zuba u donjoj vilici, uzrokovanih teskobom. Nakon analize ortopan snimka uočen je impaktiran donji desni drugi premolar koji je uzrokovao resorpciju mezijalnog korena prvog molara. Plan terapije bilo je vađenje zuba 46 i postavljanje bravice na impaktirani drugi premolar. Sledeća faza je ortodontska ekstruzija zuba 45, njegovo dovođenje u okluzalnu ravan uz dalju ortodontsku terapiju. Ortodontski tretman impaktiranih zuba fiksnim ortodontskim aparatom daje odlične terapijske rezultate. Uspešnost terapije zavisi, pre svega, od adekvatnog planiranja od samog početka, kao i saradnje i zajedničkog rada specijalista oralne hirurgije i ortodoncije.
Ključne reči: impaktirani donji premolar; ortodontska ekstruzija; vađenje zuba; resorpcija korena zuba

UVOD

Impaktirani donji premolari nisu tako retka pojava u svakodnevnoj stomatološkoj praksi. Nakon donjih i gornjih umnjaka i gornjih očnjaka oni su najčešće impaktirani zubi [1]. Mandibularni premolari niču nakon nicanja prvog donjeg molara i očnjaka, tako da često nedostaje dovoljno prostora za nicanje oba premolara, što dovodi do impakcije jednog od njih, najčešće drugog premolara [2]. Razlozi za njihovu impakciju su brojni, počevši od nedostatka prostora, ektopičnog položaja zametka, prisustva prepreke (mlečni zub, tumor, ožiljno tkivo) koja se našla na putu nicanja premolara, prisustva prekobrojnih zuba ili odontoma. Neka od sistemskih i genetskih oboljenja mogu uticati na pojavu impakcije mandibularnih premolara; pre svega, misli se na kleidokranijalnu displaziju, osteoporozu, Daunov sindrom, hipotiroidizam i hipopituitarizam [3, 4, 5].

Tretman impaktiranih mandibularnih premolara je različit u zavisnosti od položaja zuba, dubine impakcije, odnosa sa okolnim zubima, kao i toga da li je i kakva ortodontska terapija planirana. Način terapije obuhvata timski rad ortodonta koji pravi plan lečenja i oralnog hirurga sa kojim je obavljena konsultacija pre hirurške intervencije, kojom će se omogućiti pristup impaktiranom zubu. Postupak podrazumeva hirurško oslobađanje krunice impaktiranog premolara, lepljenje ortodontske bravice, uz dalji ortodontski tretman fiksnim aparatom. Jedan od uslova za uspešnu terapiju jeste i to da nagib impaktiranog premolara ne sme biti veći od 45° [6].

U ovom slučaju terapija je podrazumevala hirurško oslobađanje impaktiranog drugog donjeg premolara uz kasniji ortodontski tretman.

PRIKAZ BOLESNIKA

Pacijentkinja (22) javila se kod ortodonta na pregled zbog rešavanja problema nepravilnog položaja zuba (teskoba) u donjoj vilici (Slika 1). Nakon analize ortopan snimka utvrđeno je da je desni premolar u donjoj vilici impaktiran, sa sumnjom da postoji resorpcija mezijalnog korena prvog molara koji je u kontaktu sa impaktiranim zubom. Takođe, primećen je i nedostatak prvog premolara u trećem kvadrantu, kao i prisustvo neizniklog umnjaka u četvrtom kvadrantu (Slika 2). Pacijentkinja je potpuno zdrava i u stomatološkoj anamnezi negira prethodne ekstrakcije zuba i bilo kakve ortodontske intervencije. Takođe, ne navodi postojanje bolova ili neugodnosti u orofacijalnoj regiji.

Ortodont je pacijentkinju zatim poslao na pregled kod oralnog hirurga. Oralni hirurg je nakon kliničkog pregleda, analize ortopana i konsultacije sa ortodontom napravio plan terapije, koji je podrazumevao kombinovani hirurško-ortodontski tretman. S obzirom na to da je impaktirani drugi premolar bio u vertikalnom položaju, planirana je njegova ortodontska ekstruzija uz vađenje prvog molara. Prvi molar će biti ekstrahovan prvenstveno zbog resorpcije njegovog mezijalnog korena od strane impaktiranog drugog premolara, te zbog pravljenja mesta za smeštanje premolara.

Nakon što je prethodno postavljen fiksni ortodontski aparat obavljena je oralno-hirurška intervencija u lokalnoj anesteziji. Pažljivo je podignut ugaoni sulkusni režanj pune debljine od očnjaka do drugog molara, pri čemu se vodilo računa o blizini bradnog živca (n.mentalis). Zub 46 je pažljivo izvađen kleštima, pri čemu je uočena resorpcija središnjeg dela njegovog mezijalnog korena, nastalog usled pritiska krunice impaktiranog premolara 45. Zatim je izvađen i vrh mezijalnog korena prvog molara (Slika 3). Ekstrakcijom zuba 46 eksponirana je cela krunica impaktiranog drugog premolara (Slika 4). Dodatno je diskretno oslobođena kost oko krunice zuba 45 karbidnim borerima uz konstantno hlađenje borera sterilnim fiziološkim rastvorom. Time je omogućeno lepljenje bravice (Slika 5) i dodatno olakšan put nicanja impaktiranom zubu 45. Rana je isprana fiziološkim rastvorom i ušivena pojedinačnim šavovima, koji su uklonjeni nakon sedam dana. Pacijentkinji su dva dana ranije propisani antibiotici (Dovicin 100 mg, sedam dana), za bržu resorpciju edema i hematoma dat je Chymoral Forte 5 dana i analgetici koje je koristila po potrebi.

Dalji ortodontski tretman se tokom narednih šest meseci zasnivao na ekstruziji impaktiranog premolara, kao i njegovom postavljanju na mesto u zubnom luku i dostizanju okluzalne ravni (Slika 6). Ujedno je korigovana i teskoba zuba u donjoj vilici uopšte. Ortodontska terapija će u potpunosti biti završena nakon nicanja umnjaka u četvrtom kvadrantu, kojim će se u potpunosti postići stabilna okluzija u bočnoj regiji.

DISKUSIJA

Podaci o problemima impaktiranih donjih premolara nisu prisutni u literaturi u većoj meri bez obzira na to što je učestalost impakcije tih zuba kod pacijenata prilično velika [7]. Princip lečenja ovakvih zuba je multidisciplinaran, uz saradnju ortodonata sa oralnim i maksilofacijalnim hirurzima, dečjim stomatolozima, a nekada i endodontistima [8]. Kakav način terapije će biti odabran zavisi od više faktora, pre svega od postojanja dovoljno prostora za nicanje impaktiranog zuba, dubine impakcije, stepena formiranosti korena, potrebe za ekstrakcijom prvog molara, dužine trajanja terapije, prisustva keratinizovane gingive. Osim njih, značajan uticaj imaju i opšte zdravstveno stanje pacijenta, stanje zuba i oralne higijene, kao i funkcija i okluzija zuba [9, 10]. Andreasen [1] preporučuje da hirurško eksponiranje impaktiranih premolara treba biti ograničeno samo na slučajeve kada angulacija zuba nije veća od 45° i u gornjoj i donjoj vilici. Međutim, u praksi se mogu sresti slučajevi horizontalne impakcije mandibularnih premolara koje su uspešno sprovedenom hirurško-ortodontskom terapijom postavljeni na svoje mesto u zubnom nizu [8].

U slučajevima kada se na osnovu analize OPT snimka i kliničkog pregleda dijagnostikuje nedostatak prostora za nicanje impaktiranih mandibularnih premolara, obavezni su redovni kontrolni pregledi, zatim ekstrakcije mlečnih zuba i praćenje položaja stalnih premolara. Ukoliko je potrebno, vrši se hirurško oslobađanje krunice zuba, sa ortodontskom vučom ili bez nje, ili repozicija (autotransplantacija). Međutim, nekada nije moguće na bilo koji način impaktirani mandibularni premolar dovesti na njegovo mesto u zubnom nizu i tada se vrši hirurško vađenje tog zuba [11].

Ukoliko impaktirani mandibularni premolar želimo da smestimo na svoje mesto u zubnom nizu, primenjuje se ortodontska terapija zuba, koja se može podeliti u tri faze. Prva faza obuhvata početak ortodontske terapije hirurški eksponiranog zuba u trajanju od dva do pet meseci, u zavisnosti od vrste anomalije. Druga faza nastupa od početka vuče zuba do njegovog smeštanja u zubni luk i traje od 12 do 18 meseci. Treća faza je završetak ortodontske terapije, kada je zub na svom mestu u luku. S tim da se obično planira još oko 10–18 meseci za potpuni završetak ortodontske terapije [12].

Jedna od mogućih komplikacija koje srećemo kod impaktiranih mandibularnih premolara je i pojava razvojne ciste odontogenog porekla [3]. Ove ciste su česta pojava kod impaktiranih, retiniranih zuba ili kod zuba u nicanju. Pošto ne daju nikakvu ili daju slabu simptomatologiju, obično se dijagnostikuju slučajno tokom rutinskog pregleda ili radiografije. Ove cistične lezije dostižu često velike dimenzije koje mogu dovesti do mobilnosti i migracije okolnih zuba ili resorpcije njihovih korenova. Zbog toga je neophodno njihovo uklanjanje u celosti (cistektomija) uz vađenje impaktiranog zuba [13]. Nekada se u cilju zadržavanja zuba vrši marsupijalizacija uz dekompresiju i fenestraciju cističnog zida [14], uz oslobađanje impaktiranog zuba i nastavlja ortodontski tretman.

Sve je veći broj mladih pacijenata sa problemom impaktiranih i drugih stalnih zuba (ne samo umnjaka) i teži se uspešnoj korekciji postojeće ortodontske anomalije, što predstavlja prilično složen i dugotrajan proces. Za postizanje uspeha od ključne važnosti je pažljivo i temeljno planiranje terapije, kao i dobra saradnja oralnog hirurga i ortodonta. Timski rad, redovni kontrolni pregledi uz dobru saradnju pacijenta na kraju daju odlične rezultate u lečenju ovakvih ortodontskih malformacija.

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Various methods of 3D and Bio-printing

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SUMMARY

There is growing need for synthetic tissue replacement materials designed in a way that mimic complex structure of tissues and organs. Among various methods for fabrication of implants (scaffolds), 3D printing is very powerful technique because it enables creation of scaffolds with complex internal structures and high resolution, based on medical data sets. This method allows fabrication of scaffolds with desired macro- and micro-porosity and fully interconnected pore network. Rapid development of 3D printing technologies has enabled various applications from the creation of anatomical training models for complex surgical procedures to the printing of tissue engineering constructs. The aim of current investigations was to develop compatible printers and materials (bioinks) to obtain biomimetic scaffolds, which allow printing of living cells without significant loss of cell viability. The advanced level of such printing assumes "*in situ*" printing, i.e. printing cells and biomaterials directly onto or in a patient that will reduce recovery time.
Keywords: 3D printing; bio printing; scaffolds, biomaterials

INTRODUCTION

Tissue engineering strategy typically involves combination of cells and bioactive factors with a 3D scaffold to form useful construct for implantation [1, 2]. Ideally, scaffold should be completely resorbed during tissue integration. Biomaterial scaffolds should mimic important aspects of targeted tissue, restoring their function and providing an environment suitable for cell differentiation and proliferation [3, 4]. Traditional techniques used for production of such scaffolds are gas foaming, solvent casting, fiber bonding, phase separation, particulate leaching, and freeze drying techniques that provide macroscale scaffold features but often lack the complexity of native tissue [5].

Fabrication methods that enable production of complex geometries have significant advantages since they provide production of scaffolds of irregular shape that can perfectly fit the defect geometry. Besides, they can also mimic tissue complexity through precise positioning of multiple materials and cell types. As it is known, scaffolds should provide not only macroscale structural design, but also microscale features necessary for cellular sophisticated control over fabrication of a new tissue. Recently, 3D fabrication or rapid-prototyping technology has become popular and accessible, allowing everyday investigations of potential new fabrication techniques with better geometric accuracy on the macro and micro scale level [6, 7]. Those investigations have opened door to innumerable approaches of scaffolds engineering such as high-resolution imaging and 3D printing technology known as laser sintering which was successfully used to create functional jawbone replacement [8]. This method has enabled creation of articulated joints, cavities that promote muscle attachment, and grooves to guide nerve and vein regrowth, and also reduced surgical preparation and accelerated recovery. In addition, designed vasculature may enable creation of larger constructs useful for nutrient transport for tissue growth. Functional tissue constructs could also be applied as a diagnostic tool for drug testing or other therapeutic procedures.

Currently used 3D biofabrication printing methods can be divided into acellular techniques which include stereolithography (SLA), powder-fusion printing (PFP) and fused deposition modeling (FDM)), and bioprinting of cellularized constructs that can be inkjet-based, extrusion-based, or laser assisted (LAB)) [8].

ACELLULAR SCAFFOLD FABRICATION

Rapid prototyping techniques use multi-axis positioning systems and one of various methods to generate a 3D construct through subsequent layer fabrication (extrusion, deposition, solidification, polymerization, sintering or binding using many other methods) [8, 9]. First step is creating a model in a computer-aided design (CAD) program and export it into the file format that describes the volume or surface mesh in 3D space such as *.stl (stereolithography), *.obj (object), or *.amf (additive manufacturing file). Second step is translation of the 3D data into slices to be patterned by the printer program using the

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program generally known as a 'slicer'. These techniques enable user to configure algorithm that determines pattern used to fill the layers and then the program calculates necessary parameters such as extrusion speed, cure time, or laser speed to accurately fill the pattern.

Previously, these techniques were adapted to mold casting, but recent rapid development increased their versatility and precision. Nowadays techniques are able to create scaffolds that fully mimic macroscale organs geometry and print layers with thickness less than 20 μm allowing complete reproduction of the tissue microarchitecture. Techniques with higher precision are currently under investigation to enable reproduction of smaller tissue features such as hepatic lobules and kidney nephrons.

Stereolithography (SLA)

SLA techniques use deflected laser beam or projected light source to cure and harden given areas of photopolymer at the surface of some material (Figure 1) [8, 10]. Various photopolymers with suitable viscosity and ability to harden can be used in construct creation with SLA. Cooke used SLA to fabricate 3D scaffolds for bone tissue engineering using biodegradable polymers, like diethyl fumarate and polypropylene fumarate [10]. Also, photo-curable ceramic acrylate suspension was used to form a construct of cancellous bone and bone scaffolds using hydroxyapatite [7].

The disadvantage of SLA methods is limited resolution by the diameter of laser beam (about 250 μm), although small-spot laser systems and digital light processing projection produced features of about 70 μm. These techniques can also be used to design hydrogel scaffolds from natural and synthetic polymers that expand in water and are significantly less rigid than traditional SLA constructs. Hydrogels have become popular as tissue engineering biomaterials due to their high water content and mechanics similar to soft tissue. Some researchers use this technique for creation of 2- hydroxyethyl methacrylate scaffolds using photolithography for formation of patterns from non-swollen prepolymer, which were then hydrated and seeded with cells [11]. SLA has also been used to make molds that are used to cast negative replicas of the printed molds. Chu et al. made printed mold of a mandible generated using CAD program and data from computed tomography imaging. The mold was filled with a hydroxyapatite/acrylate mixture and heated to cure the scaffold [12].

Accordingly, SLA seems to be a versatile and attractive technique for creating tissue-engineering scaffolds because of its precision and increasing availability of biologically relevant photopolymers.

Powder-fusion printing (PFP)

PFP uses granular materials (plastic, resin, or metal) for printing that are selectively bound together (Figure 2) [8, 13]. In selective laser sintering-melting (SLS/SLM), plastic or metal granules are sintered together by a laser beam that is directed across the powder bed, to increase local

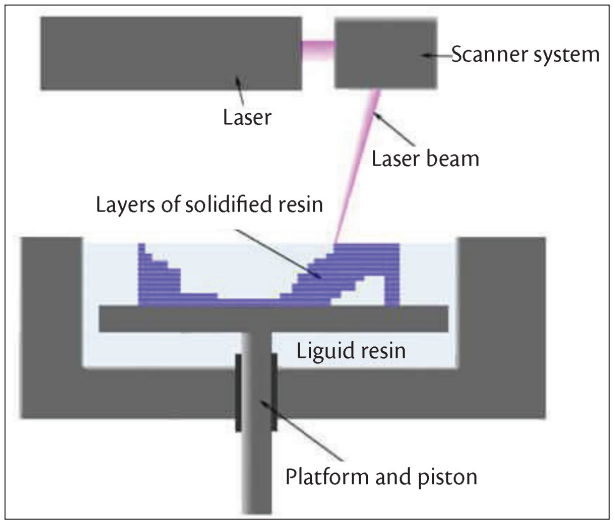

Figure 1. SLA 3D printer used for printing resin-based photopolymers
Liquid material solidifies by a high-powered laser or light source, "activating" the photopolymerization reaction. If laser is used to "draw" the object's layers, this method is known as pure SLA. If the method is based on te digital light projection of entire slice of the object this process is known as digital light processing (DLP).
Slika 1. 3D štampač SLA koji se koristi za štampanje fotopolimera na bazi smola
Tečni materijal se očvršćava pomoću laserskog ili svetlosnog izvora velike snage, aktiviranjem reakcije fotopolimerizacije. Ako se koristi laser za „crtanje" slojeva objekta, ova metoda je poznata kao čista stereolitografija. Ako je metoda zasnovana na projekciji digitalne projekcije čitavog objekta, ovaj proces je poznat kao proces digitalne obrade svetlosti (DLP).

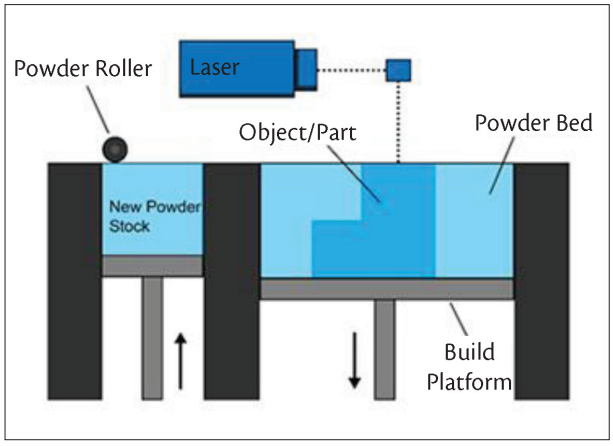

Figure 2. Powder Bed Fusion printer: A layer, typically 0.1mm thick material is spread over the built platform, then laser fuses the first layer or first cross section of the model. A new layer of powder is spread across the previous layer using a roller. Further layers or cross sections are fused and added. The process repeats until the entire model is created. Loose, un-fused powder remains in position but is removed during post processing.
Slika 2. Štampač praškastog fuzionog sloja: sloj materijala, tipično debljine 0,1 mm, nanosi se preko platforme, i laserski lepi prvi sloj ili prvi presek modela. Novi sloj praha se širi preko prethodnog sloja pomoću valjka. Dalji slojevi ili preseci se spajaju i dodaju. Proces se ponavlja sve dok se ne kreira ceo model. Neiskorišćeni prah ostaje na poziciji, ali se uklanja kasnije tokom obrade.

temperature influencing particle fusion in the heated area along the laser path. 3D scaffolds are generated by layer-by-layer deposition of the powder. After fabrication, unfused powder is removed and the resulting part is mechanically strong construct with carefully designed geometry and porosity. As in SLA, the resolution of SLS printing depends on the spot size of the laser beam and the size of powder particles. Typical laser-based systems have minimum features of about 400 μm, with minimum void size of about 50 μm. SLS techniques have also been developed to fabricate constructs with various biopolymers used in a wide variety of medical implants.

Scaffolds can also be made from granular material by binding the particles with solvents or adhesives whereby

they are built layer-by-layer. Also, scaffolds can be fabricated from natural biopolymers and polysaccharides like gelatin, dextran, and starch. Microporous structures can be achieved with the addition of porogens and particulate leaching. For example, Simpson et al. fabricated porous poly (lactic-co-glycolic) acid scaffold using PFP and precisely reproduced the shape of an entire human finger phalanx [14]. These porous structures were also investigated from the aspect of cell attachment, growth, and matrix deposition.

Although PFP is limited to powdered materials, its advantage is capability to fabricate scaffolds from several materials such as titanium and magnesium that are not readily printable with other techniques. PFP is particularly suitable for bone and other rigid tissues scaffolds because bound or fused material creates constructs of superior mechanical properties. In addition, some materials naturally found in bone such as tricalcium phosphate can also be printed using PFP techniques, allowing creation of complex scaffold shapes, including in advance designed interconnected porosity. The resolution and minimum pore size are limited by the powder characteristics, and additional sintering is necessary to solidify parts that contain cracks and other damages. The focus of current research is on developing new materials for PFP and refinement of printing parameters to improve scaffold surface design.

Fused deposition modeling (FDM)

FDM techniques enable useful platform for scaffolds creation by using precise xyz positioning system to direct the position of a nozzle during material deposition [8, 15]. The material is deposited in layers and solidified into a previously defined shape. Traditional SFF printers are frequently used for rapid prototyping by using a small diameter polymer feedstock of acrylonitrile butadiene styrene which is forced through the nozzle heated to temperatures higher than 200°C.

Biodegradable polymers used in tissue engineering typically melt at lower temperatures and can be printed at more moderate temperatures (60-100 °C). Using this method it is possible to produce precise lattice structure, if temperature is precisely controlled and optimized with speed parameters during generation of filament with required accuracy. Newer generations of FDM systems use heated reservoir for extrusion of polymer pellets rather than fibers. Scaffolds produced by this technique from alginate and PCL implanted in mice have shown enhanced cartilage and collagen formation over a 4-week implantation [8].

Decreasing nozzle size and layer height increases x–y and z resolution, leading to significantly slower extrusion rates. Theoretical resolution is limited by the precision of the linear motion system (motors, gears, timing belts, and leadscrews) and retention properties of extruded material. Although FDM techniques enable the achievement of high degree of positional accuracy in the xy plane, their substantial limitation is in disability to print overhanging or unsupported parts because there is no supporting

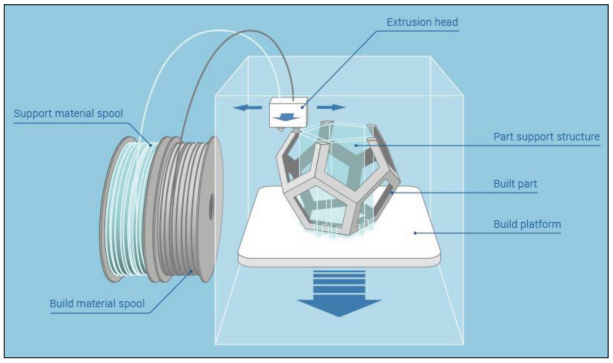

Figure 3. Sheme of fused deposition modelling technique (source: www.additively.com)
Slika 3. Šema procesa fuzione depozicije (izvor: www.additively.com)

material from previous layers. Therefore, hardening during cooling or cross-linking after extrusion is essential for satisfied support of subsequent layers. Also, this drawback can be solved with introducing filament of support material during the process of printing, usually through additional extruder (Figure 3).

Recent improvements in hydrogel rheological properties enable printing of these materials using FDM. For example, Hong et al. created printable hydrogel using a network of PEG and alginate with silicate nano-platelets [16]. These gels possessed zero-shear viscosity above 10 kPa·s, enabling shape retention after printing and a shear thinning that facilitated extrusion. The size and accuracy of printed hydrogel construct are dependent on the volume contained in the syringe and rheological properties of the hydrogel. Viscosity plays a key role in construct accuracy, because high-viscosity materials possess structural rigidity that is important for support of extruded successive layers, and secondary cross-linking step is typically used to lock the printed shape and improve mechanical properties of these constructs.

Extrusion-based printers typically use pneumatic pressure or a motor actuated plunger for material deposition. Pneumatic systems simplify control of the applied force to extruded material. The system should be calibrated for each material with adjustments of the nozzle size, nozzle geometry (tapered tip, cylindrical needle, and length), and gas pressure.

FDM seems to be one of the most versatile printing techniques for creation of biomimetic scaffolds due to its ability to make multilayered constructs built from various materials and print soft biomaterials like hydrogels (Figure 4). Scaffolds printed by this technique may exhibit anisotropic mechanical properties that can be useful for creating scaffolds with intended alignment such as ligament or tendon.

BIOPRINTING

Bioprinting belongs to additive manufacturing techniques for creation of the cell-based scaffolds [17]. These techniques are presumably adapted for printing with cells at the same time as material, since they have minimal impact on the cell viability and function. Biological materials used for printing should match natural environment

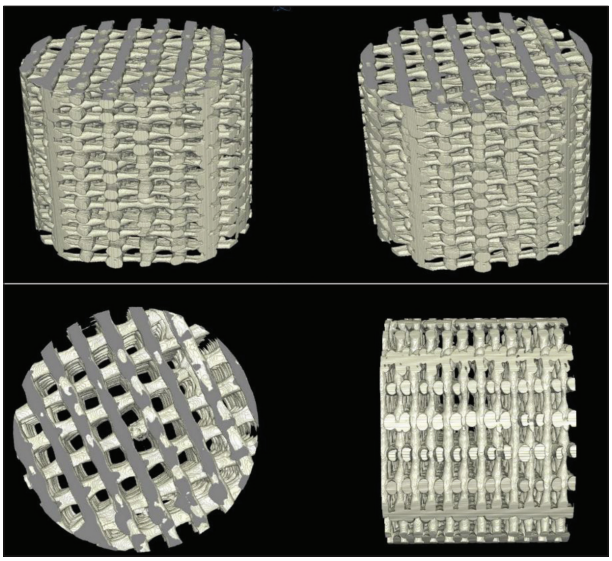

Figure 4. Micro CT volume reconstruction, example of porous bone scafold structure, cilinder of 10 mm in hight and 10 mm diameter. Cilinder is made by extrusion printing (FDM) and has ideal porosity, trabecular thickness, pore diameter and connectivity of pores for bone scafold model.
Slika 4. Mikro CT zapreminska rekontrukcija, primer poroznog koštanog skafolda, visine 10 mm i prečnika 10 mm. Cilindar je napravljen ekstruizonim štampanjem (FDM) i ima idealnu poroznost, trabekularnu debljinu, prečnik pora i povezanost pora, kao skafold za koštano tkivo.

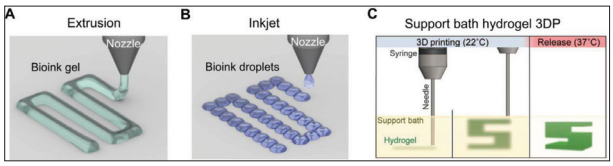

Figure 5. Extrusion-based 3D bioprinting
(A) For extrusion-based bioprinting, material is selectively guided onto a platform via pressurized emission through the nozzle. The material, or 'bioink', is composed of an ECM-like biomaterial, cells and soluble factors.
(B) For inkjet-based bioprinting, droplets of bioink are distributed across a surface to form a patterned layer.
(C) For support bath hydrogel 3DP, biomaterial is extruded into the support hydrogel material. At 22°C, the hydrogel bath is stable enough to support the extruded print material, but at 37°C, the hydrogel bath transitions into the more liquid state to release the 3D printed object. The support bath allows formation of complex structures.
Slika 5. Ekstruziono 3D bio-štampanje
(A) Za ekstruziono bio-štampanje materijal se selektivno usmerava na platformu putem emitovanja pritiska kroz mlaznicu. Materijal, ili 'bioink', sastoji se od biomaterijala sličnih ECM-u, ćelija i rastvornih faktora.
(B) Za bio-štampanje na bazi inkdžeta kapljice bioinka se raspoređuju preko površine kako bi se formirao sloj.
(C) Za hidrogelom potpomognuto 3DP biomaterijal se ekstrudira u pomoćni hidrogel. Na 22 °C hidrogelno kupatilo je dovoljno stabilno da podrži ekstrudirani štampani materijal, ali na 37 °C hidrogelno kupatilo prelazi u tečno stanje da oslobodi 3D štampani predmet. Pomoćno kupatilo omogućava formiranje složenih struktura.

of the host tissue to support function of those cells. Additionally, cells should be able to overcome shear stress during the printing process and survive in real non-physiological conditions of the printing regime [8].

Bioprinting techniques are classified into the three categories: microextrusion, lasser-assisted bioprinting (LAB) and inkjet-based bioprinting. Among them, inkjet bioprinting is the most promising for the creation of complex architectures, successfully mimicking native tissue and organs. In inkjet-bioprinting, bioink droplets are deposited onto the substrate that gels to form polymeric structures, while microextrusion bioprinting uses mechanical extruder to deposit bioink. Additionally, extrusion-bioprinting is useful for high cell density, due to its easier processing, but it is a slower than drop-based bioprinting. LAB requests a picoliter (pL) resolution through which cells and liquid materials can be printed. This printing method is rapidly growing and it is promising for the fabrication of tissue-like constructs.

Extrusion bioprinting

Extrusion-bioprinting is one of the most economical techniques for rapid prototyping (Figure 5) [8, 17]. It typically includes pressure or screw/plunger-actuated dispensing of a fluid containing cells and/or biomaterials. It should provide shear thinning enabling minimal resistance under flow and quick chemical or physical cross-link after extrusion to support successive layers. This technique allows accurate deposition of the material and fabrication of complex patterned structures, including the use of multiple cell types, enabling accelerated growth and new tissue formation. Increasing print resolution and print speed are desirable in extrusion-bioprinting. Additionally, by the modification of printing mechanics, printing time can be diminished and coextrusion of multiple materials can be permitted. The main disadvantage is relatively long fabrication time to achieve high resolution in complex structures. This method enables successful fabrication of clinically relevant scaffolds for tissue engineering, because it is ideally adjusted for biological materials due to its ability to deposit multiple materials with wide-ranging properties. Extrusion bioprinted scaffolds are typically soft, due to their high water content that makes them limited to soft tissues application.

Laser-assisted bioprinting (LAB)

LAB, or biological laser printing, is a group of laser techniques that use laser energy to facilitate densification of scaffold materials (Figure 6) [8, 17]. One type of LAB uses laser pulse (laser based direct writing (LDW)) for local heating a slide with an energy-absorbing layer and solution of cells. The laser pulse induces sublimation or evaporation of material, expelling the solution of cells on the opposite side and precisely depositing them on the substrate. This method includes laser-induced forward transfer and matrix-assisted pulsed laser evaporation, which can be used for deposition of fibroblasts, keratinocytes, human mesenchymal stem cells, various cancer cells and biopolymers.

As lasers technique allows high precision, this method is suitable for bioprinting of the smallest details of native tissues and organs. This technique allows direct printing of cells, but with several limitations, like detrimental effect on cell survival and their long-term behavior.

Inkjet bioprinting

Inkjet bioprinting enables precious deposition of cells and biomaterials, using some advances of 2D inkjet printing to create 3D scaffolds [8, 18]. In this method a limited volume of fluid is falling into the precise pattern specified by the corresponding software. One of the most important

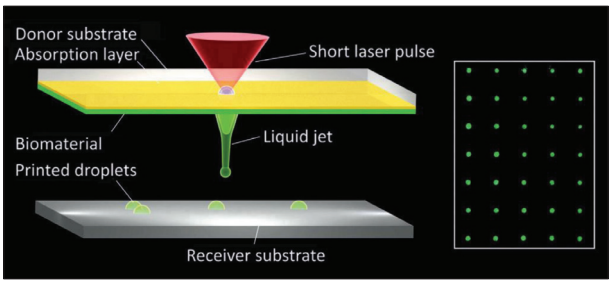

Figure 6. Laser printing of vital cells embedded in hydrogel. The focused laser pulses evaporate an absorption layer locally. The vapor pressure propels the subjacent biomaterial as a liquid jet toward the receiver substrate. The jet lasts for a few hundred microseconds and remains a droplet. The microscopic image (right) shows droplets with fluorescent cells.
Slika 6. Lasersko štampanje vitalnih ćelija umetnutih u hidrogel. Fokusirani laserski puls dovodi do lokalnog isparavanja apsorpcionog sloja. Pritisak pare propagira donji biomaterijal kao mlaz tečnosti prema supstratu prijemniku. Taj mlaz traje nekoliko stotina mikrosekundi i ostaje kapljica. Mikroskopska slika (desno) pokazuje kapljice sa fluorescentnim ćelijama.

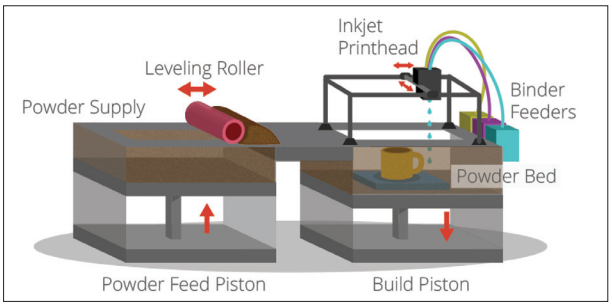

Figure 7. A binder is firstly jetted and selectively sprayed into a powder bed. When one layer is printed, the powder bed drops incrementally and a roller or blade applies and flattens the powder over the surface of the bed, prior to the next pass of the jet heads, with the binder for the subsequent layer to be formed and fused with the previous layer.
Slika 7. Vezivno sredstvo se prvo izbacuje kroz mlaznicu i selektivno prska na rezervoar praha. Kada se odštampa jedan sloj, rezervoar praha postepeno opada, a valjak ili sečivo poravnava prah pre sledećeg prolaza mlaznih glava, sa vezivnim sredstvom za sledeći sloj koji se formira i kondenzuje sa prethodnim slojem.

advantages of this technique is the speed at which it can construct scaffolds with complex 3D architecture. This high speed limits the number of polymeric materials that can be used for bio-printing, since their gelation time has to be greater or equal to the drop deposition time.

Inkjet bio-printers can be adjusted to print materials at increased resolutions and speeds. They use thermal or piezoelectric energy to deposit droplets of solution into desired patterns and consist of one or many ink chambers with multiple nozzles with corresponding piezoelectric or heating components. A short pulse of current is used to actuate the component and eject a droplet of ink. In thermal bio-printers, there is often a strong increase of temperature in local spots, inducing formation of vapor bubbles and collapsing, leading to ejecting ink droplets onto the substrate. In piezoelectric inkjet printing, piezocrystals induce pressure increase, which further influence the droplet ejection. Deposition from the nozzle onto the printing surface happens when an electric charge induces vibration in the crystals, and vibration propagates to printing surface. It has been shown that heat and mechanical stresses during thermal bioprinting cause decrease of cell viability. Some researchers use this method for printing of retinal ganglion and glia cells isolated from adult central nervous system without causing an adverse effect on cell viability, while some of them succeeded in use of thermo-sensitive gels by modifying cartridge of commercially available inkjet printer to create multilayered scaffolds [8].

The main disadvantage of inkjet printing is request for biological agents to be in a liquid state, to allow deposition. Deposited droplets then solidify into the required geometry, through cross-linking based on physical, chemical, pH, or ultraviolet methods. Due to chemical cross-linking, many natural materials frequently change their chemical properties. In addition, some cross-linking mechanisms induce decrease of cell viability and functionality (Figure 7).

Although inkjet bioprinting enables encapsulation of live cells, their concentration has to be relatively low in order to form cohesive droplets and prevent clogging of the nozzle. Despite numerous disadvantages, this method has a great potential due to its low cost, high resolution, and high compatibility with many biomaterials. Additionally, these printers enable accurate deposition of fine droplets with precise volume to create high-resolution scaffolds with cells intact. Droplet size can be modulated from 1 to 300 pL with deposition rates from 1 to 10,000 droplets per second. Therefore, this method enables scaffolds creation with accuracy within 100 μm, which is very promising for creating complex scaffolds. Although it cannot produce very tall structures, influenced by the typical mechanical properties of the gel inks, due to its ability to print multiple structures and cell types it is very convenient for printing complex tissues with great accuracy.

INSTEAD OF CONCLUSION: EXPECTED FATE OF THESE METHODS IN THE FUTURE

Adaptation of current 3D printing methods for biological applications has enormous importance for future fabrication of tissue grafts and artificial organs. Besides tissue engineering, 3D printing is also used in the area of drug delivery, analysis of chemical and biological agents and organ-on-a-chip devices [19].

Despite its huge potential in regenerative strategies, the main challenges are related to necessity of improved resolution, increased speed and printing that enables cells survival [18]. Current efforts in improvement of printing resolution in lithography assume the development of methods like electron beam lithography and multi-photon absorption polymerization, because these methods are suitable for creation of scaffolds with extremely precise feature sizes, of the order of only of tens of nanometers [20].

Materials used for 3D bioprinting must meet the following criteria: should be biocompatible, support cell growth and differentiation and retain its shape long enough to preserve scaffold integrity until solidification locks in scaffold geometry. The most commonly used materials for such purposes are collagen, gelatin, hyaluronic acid, alginate, modified copolymers, and photo-polymerizablemacromers [21].

For design of complex scaffolds that mimic tissue, additional research is necessary for accurate mapping of complex tissues to be able to make well-reproduced scaffolds with required structures and biological properties. One of the main challenges in future in 3D printing is

direct „*in situ*" bioprinting, or printing cells and biomaterials directly onto or in a patient. Some recent research showed capabilities of bioprinting directly into wounds or burn defects [22]. Further improvements of the printing speed and resolution are needed for „*in situ*" printing that will enhance tissue regeneration and reduce patients recovery time.

REFERENCES

1. Amini AR, Laurencin CT, Nukavarapu SP. Bone Tissue Engineering: Recent Advances and Challenges. Crit Rev Biomed Eng. 2012; 40(5):363-408. [PMID: 23339648]
2. Li JJ, Kaplan DL, Zreiqat H. Scaffold-based regeneration of skeletal tissues to meet clinical challenges. J Mater Chem B Mater Biol Med. 2014; 2(42):7272-306. [DOI: 10.1039/c4tb01073f]
3. Jokanović V, Čolović B, Marković D, Petrović M, Soldatović I, Antonijević D, et al. Extraordinary biological properties of a new calcium hydroxyapatite/poly(lactide-co-glycolide)-based scaffold confirmed by in vivo investigation. Biomed Tech. 2017; 62(3):295-306. [DOI: 10.1515/bmt-2015-0164] [PMID: 27285125]
4. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials. 2000; 21(24):2529-43. [DOI: 10.1016/S0142-9612(00)00121-6] [PMID: 11071603]
5. Zhu N, Chen X. Biofabrication of Tissue Scaffolds. In: Pignatello R, editor. Advances in Biomaterials Science and Biomedical Applications. Rijeka: InTech; 2013. [DOI: 10.5772/54125]
6. Ventola CL. Medical Applications for 3D Printing: Current and Projected Uses, Pharmacy and Therapeutics. P T. 2014; 39(10):704-11. [PMID: 25336867]
7. Leukers B, Gülkan H, Irsen SH, Milz S, Tille C, Schieker M, et al. Hydroxyapatite scaffolds for bone tissue engineering made by 3D printing. J Mater Sci Mater Med. 2005; 16(12):1121-4. [DOI: 10.1007/s10856-005-4716-5] [PMID: 16362210]
8. Sears NA, Seshadri DR, Dhavalikar PS, Cosgriff-Hernandez E. A Review of Three-Dimensional Printing in Tissue Engineering. Tissue Eng Part B Rev. 2016; 22(4):298-310. [DOI: 10.1089/ten.teb.2015.0464] [PMID: 26857350]
9. Peltola SM, Melchels FP, Grijpma DW, Kellomäki M. A review of rapid prototyping techniques for tissue engineering purposes. Ann Med. 2008; 40(4):268-80. [DOI: 10.1080/07853890701881788] [PMID: 18428020]
10. Cooke MN, Fisher JP, Dean D, Rimnac C, Mikos AG. Use of stereolithography to manufacture critical-sized 3D biodegradable scaffolds for bone ingrowth. J Biomed Mater Res B Appl Biomater. 2003; 64(2):65-9. [DOI: 10.1002/jbm.b.10485] [PMID: 12516080]
11. Yu T, Ober CK. Methods for the topographical patterning and patterned surface modification of hydrogels based on hydroxyethyl methacrylate. Biomacromolecules. 2003; 4(5):1126-31. [DOI: 10.1021/bm034079m] [PMID: 12959574]
12. Chu TM, Hollister SJ, Halloran JW, Feinberg SE, Orton DG. Manufacturing and characterization of 3-D hydroxyapatite bone tissue engineering scaffolds. Ann N Y Acad Sci. 2002; 961:114-7. [PMID: 12081877]
13. Yang S, Leong KF, Du Z, Chua CK. The Design of Scaffolds for Use in Tissue Engineering. Part II. Rapid Prototyping Techniques. Tissue Eng. 2002; 8(1):1-11. [DOI: 10.1089/107632702753503009] [PMID: 11886649]
14. Simpson RL, Wiria FE, Amis AA, Chua CK, Leong KF, Hansen UN, et al. Development of a 95/5 poly(L-lactide-co-glycolide)/hydroxylapatite and beta-tricalcium phosphate scaffold as bone replacement material via selective laser sintering. J Biomed Mater Res B Appl Biomater. 2008; 84(1):17-25. [DOI: 10.1002/jbm.b.30839] [PMID: 17465027]
15. Zein I, Hutmacher DW, Tan KC, Teoh SH. Fused deposition modeling of novel scaffold architectures for tissue engineering applications. Biomaterials. 2002; 23(4):1169-85. [DOI: 10.1016/S0142-9612(01)00232-0] [PMID: 11791921]
16. Hong S, Sycks D, Chan HF, Lin S, Lopez GP, Guilak F, et al. Printing of Highly Stretchable and Tough Hydrogels into Complex, Cellularized Structures. Adv Mater. 2015; 27(27):4035-40. [DOI: 10.1002/adma.201501099] [PMID: 26033288]
17. Kang HW, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. Nat Biotechnol. 2016; 34(3):312-9. [DOI: 10.1038/nbt.3413] [PMID: 26878319]
18. Murphy SV, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol. 2014; 32(8):773-85. [DOI: 10.1038/nbt.2958] [PMID: 25093879]
19. Low KH, Leong KF, Chua CK, Du ZH, Cheah CM. Characterization of SLS parts for drug delivery devices. Rapid Prototyp J. 2001; 7(5):262-8. [DOI: 10.1108/13552540110410468]
20. Linjie L, Fourkas JT. Multiphoton polymerization. Materials Today. 2007; 10(6):30-7. [DOI: 10.1016/S1369-7021(07)70130-X]
21. Gross BC, Erkal JL, Lockwood SY, Chen C, Spence DM. Evaluation of 3D Printing and Its Potential Impact on Biotechnology and the Chemical Sciences. Anal Chem. 2014; 86(7):3240-53. [DOI: 10.1021/ac403397r] [PMID: 24432804]
22. Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. Stem Cells Transl Med. 2012; 1(11):792-802. [DOI: 10.5966/sctm.2012-0088] [PMID: 23197691]

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Različite metode 3D štampanja i bio-štampanja

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KRATAK SADRŽAJ

Danas postoji sve veća potreba za sintetičkim materijalima za zamenu tkiva dizajniranih na način koji imitira složenu strukturu tkiva i organa. Među različitim metodama proizvodnje implantata (skafolda), 3D štampanje je veoma moćna tehnika jer omogućava kreiranje skafolda sa složenim unutrašnjim strukturama i visokom rezolucijom, zasnovanim na medicinskim skupovima podataka. Ova metoda omogućava proizvodnju skafolda sa željenom makroporoznošću i mikroporoznošću i potpuno povezanom mrežom pora. Brzi razvoj tehnologija 3D štampanja omogućio je različite primene – od kreiranja anatomskih modela za uvežbavanje složenih hirurških procedura do štampanja konstrukata za tkivno inženjerstvo. Cilj tekućih istraživanja je razvoj kompatibilnih štampača i materijala (bio-mastila) za dobijanje biomimičnih skafolda, koji omogućavaju štampanje živih ćelija bez značajnog gubitka njihove vijabilnosti. Napredni nivo takvog štampanja pretpostavlja štampanje *in situ*, tj. štampanje ćelija i biomaterijala direktno na pacijentu ili u pacijenta, što će smanjiti vreme oporavka.

Ključne reči: 3D štampanje; bioštampanje; skafold; biomaterijali

UVOD

Strategija tkivnog inženjerstva obično podrazumeva kombinaciju ćelija i bioaktivnih faktora i 3D skafolda kako bi se dobio koristan implant [1, 2]. Idealno, skafold treba da se potpuno resorbuje tokom integracije tkiva. Skafoldi izgrađeni od biomaterijala treba da imitiraju važne aspekte ciljnog tkiva, obnavljaju njihove funkcije i obezbeđuju okruženje pogodno za diferencijaciju i proliferaciju ćelija [3, 4]. Tradicionalne tehnike koje se koriste za proizvodnju takvih skafolda su gasna pena, livenje rastvarača, vezivanje vlaknima, fazno odvajanje, čišćenje čestica i tehnike zamrzavanja, koji obezbeđuju makroskopsku strukturu skafolda, ali često nemaju osobenosti prirodnog tkiva [5].

Metode koje omogućavaju dobijanje složenih geometrija imaju značajne prednosti jer omogućavaju proizvodnju skafolda nepravilnog oblika koji se savršeno uklapaju u geometriju defekta. Pored toga, mogu da imitiraju složenost tkiva kroz precizno pozicioniranje višestrukih materijala i tipova ćelija. Kao što je poznato, skafoldi treba da obezbede ne samo makroskopski strukturni dizajn već i mikroskopske karakteristike potrebne za sofisticiranu ćelijsku kontrolu nad formiranjem novog tkiva. Odskora je 3D tehnologija ili tehnologija brzih prototipova postala veoma popularna i pristupačna, omogućavajući svakodnevna istraživanja potencijalnih novih tehnika izrade sa boljom geometrijskom preciznošću na makroskali i mikroskali [6, 7]. Ova istraživanja otvaraju vrata bezbrojnim pristupima inženjerstva skafolda, kao što je snimanje sa visokom rezolucijom i tehnologija 3D štampanja poznata pod nazivom lasersko sinterovanje, koja se uspešno koristi kao funkcionalna zamena za viličnu kost [8]. Ova metoda je omogućila formiranje zglobova, šupljina koje promovišu vezivanje mišića i žlebova za vođenu regeneraciju nerava i vena, a takođe su smanjili potrebu za hirurškom pripremom i ubrzali oporavak. Pored toga, dizajnirana vaskularna mreža može omogućiti stvaranje većih konstrukcija korisnih za transport hranljivih materija za rast tkiva. Funkcionalne konstrukcije tkiva mogu se takođe primeniti kao dijagnostički alat za testiranje lekova ili za druge terapijske procedure.

Trenutno korišćene metode štampanja mogu se podeliti na acelularne tehnike, koje uključuju stereolitografiju (SLA), štampanje fuzijom praha (PFP) i fuzionu depoziciju (FDM), i bio-štampanje ćelijskih konstrukcija, koje može biti na principu inkdžet, ekstruzije ili potpomognuto laserom (LAB) [8].

ACELULARNE METODE ZA DOBIJANJE SKAFOLDA

Tehnike brzog prototipovanja koriste višeosne sisteme za pozicioniranje i jednu od različitih metoda za generisanje 3D konstrukcija kroz sukcesivnu izradu slojeva (deponovanje ekstruzijom, očvršćavanje, polimerizacija, sinterovanje ili vezivanje koristeći mnoge druge metode) [8, 9]. Prvi korak je kreiranje modela u kompjuterskom programu (CAD) i prebacivanje u format datoteke koji opisuje površinu ili mrežnu površinu u 3D prostoru kao što su *.stl (stereolitografija), *.obj (objekat), ili *.amf (aditivno proizveden fajl). Drugi korak je pretvaranje 3D podataka u „kriške" koje će biti precrtane od strane programa za štampač, što se obavlja pomoću programa poznatog kao „slicer". Ove tehnike omogućavaju korisniku da konfiguriše algoritam koji određuje šablon koji se koristi za popunjavanje slojeva, a zatim program izračunava potrebne parametre kao što su brzina ekstruzije, vreme sušenja ili brzina lasera kako bi se tačno popunio šablon.

Prethodno su ove tehnike bile prilagođene za livenje kalupa, ali je nedavni brzi razvoj povećao njihovu svestranost i preciznost. Uz pomoć današnjih tehnika mogu se dobiti skafoldi koji u potpunosti imitiraju makroskopsku geometriju organa i štampati slojevi debljine manje od 20 μm, što omogućava potpunu reprodukciju mikroarhitekture tkiva. Tehnike sa većom preciznošću trenutno se intenzivno istražuju kako bi se omogućila reprodukcija manjih tkivnih formacija, kao što su hepatični lobuli i bubrežni nefroni.

Stereolitografija

SLA tehnike koriste skrenuti laserski zrak ili projektovani izvor svetlosti za očvršćavanje datih područja fotopolimera na površini nekog materijala (Slika 1) [8, 10]. Razni fotopolimeri sa odgovarajućim viskozitetom i sposobnošću očvršćavanja mogu

se koristiti za pravljenje konstrukata sa SLA. Kuk je koristio SLA za izradu 3D skafolda za inženjerstvo koštanog tkiva koristeći biorazgradive polimere, kao što su dietil-fumarat i poli(propilen fumarat) [10]. Takođe, fotostabilna keramička akrilatna suspenzija je korišćena da se formira konstrukcija šuplje kosti i koštanih skafolda korišćenjem hidroksiapatita [7].

Nedostatak SLA metoda je taj što je rezolucija ograničena prečnikom laserskog zraka (oko 250 μm), iako su laserski sistemi sa malim spotom i digitalna projekcija obrade svetlosti proizveli detalje veličine oko 70 μm. Ove tehnike se takođe mogu koristiti za dizajniranje skafolda od hidrogelova prirodnih i sintetičkih polimera koji bubre u vodi i znatno su manje kruti od tradicionalnih SLA konstrukata. Hidrogelovi su postali veoma popularni kao biomaterijali u inženjerstvu tkiva zbog visokog sadržaja vode i mehanike slične mekim tkivima. Neki istraživači su koristili ovu tehniku za dobijanje 2-hidroksietil-metakrilatnih skafolda koristeći fotolitografiju za formiranje šablona iz nenabubrenog prepolimera, koji su zatim hidrirani i zasejani ćelijama [11]. SLA se takođe koristi za izradu kalupa koji se koriste za livenje negativnih replika odštampanih kalupa. Ču i saradnici su napravili štampani kalup mandibule, generisan pomoću programa CAD i podataka sa snimanja računarskom tomografijom. Kalup je napunjen mešavinom hidroksiapatita i akrilata i zagrejan da bi se formirao skafold [12].

Shodno tome, izgleda da je SLA svestrana i atraktivna tehnika za dobijanje skafolda za inženjerstvo tkiva zbog svoje preciznosti i povećane dostupnosti biološki relevantnih fotopolimera.

Štampanje fuzijom praha

PFP koristi granularni materijal (plastika, guma, ili metal) za štampanje, koji se selektivno vezuje zajedno (Slika 2) [8, 13]. Kod selektivnog laserskog sinterovanja-topljenja (SLS/SLM), plastične ili metalne granule su sinterovane zajedno pomoću laserskog zraka koji je usmeren preko praha, da bi se povećala lokalna temperatura koja utiče na fuziju čestica u zagrejanom području duž puta lasera. 3D skafoldi se generišu nanošenjem praha sloj po sloj. Nakon izrade neiskorišćeni prah se uklanja, a rezultat je mehanički jaka konstrukcija sa pažljivo dizajniranom geometrijom i poroznošću. Kao i kod SLA, rezolucija SLS štampanja zavisi od veličine spota laserskog zraka i veličine čestica praha. Tipični laserski sistemi imaju minimalne karakteristike od oko 400 μm, sa minimalnom veličinom pora od oko 50 μm. SLS tehnike su takođe razvijene tako da proizvode konstrukte sa različitim biopolimerima, koji se koriste u širokom spektru medicinskih implantata.

Skafoldi se takođe mogu izrađivati od granularnog materijala vezivanjem čestica rastvaračima ili lepkom, pri čemu se grade sloj po sloj. Takođe, skafoldi mogu biti izrađeni od prirodnih biopolimera i polisaharida kao što su želatin, dekstran i skrob. Mikroporozne strukture se mogu postići dodavanjem porogena i namakanjem čestica. Na primer, Simpson i sar. su proizveli porozni skafold od poli (mlečno-ko-glikolne) kiseline pomoću PFP-a i precizno reprodukovali oblik čitave falange ljudskog prsta [14]. Ove porozne strukture takođe su istražene sa aspekta vezivanja ćelija, rasta i depozicije matriksa.

Iako je PFP ograničen na praškaste materijale, njegova prednost je sposobnost izrade skafolda iz nekoliko materijala kao što su titanijum i magnezijum, koji se ne mogu lako štampati sa drugim tehnikama. PFP je posebno pogodan za koštane skafolde i skafolde drugih čvrstih tkiva jer vezani ili spojeni materijali stvaraju konstrukte superiornih mehaničkih svojstava. Osim toga, neki materijali koji se prirodno nalaze u kosti, kao što je trikalcijum-fosfat, takođe mogu biti odštampani korišćenjem PFP tehnika, omogućavajući stvaranje složenih oblika skafolda, uključujući unapred konstruisanu međusobno povezanu poroznost. Rezolucija i minimalna veličina pora ograničeni su karakteristikama praha, a dodatno sinterovanje je neophodno za očvršćavanje delova koji sadrže pukotine i druga oštećenja. Fokus tekućih istraživanja je na razvoju novih materijala za PFP i usavršavanju parametara štampanja radi poboljšanja površinskog dizajna skafolda.

Fuziona depozicija

FDM tehnike omogućavaju korisnu platformu za kreiranje skafolda pomoću preciznog xyz sistema pozicioniranja za usmeravanje položaja mlaznice prilikom nanošenja materijala [8, 15]. Materijal se deponuje u slojevima i učvršćuje u prethodno definisanom obliku. Tradicionalni SFF štampači često se koriste za brze prototipove korišćenjem akrilatril-butadien-stirena malog prečnika, koji se ubacuje kroz mlaznicu koja se zagreva do temperature veće od 200° C.

Biorazgradivi polimeri koji se koriste u inženjerstvu tkiva uglavnom se rastvaraju na nižim temperaturama i mogu se odštampati na umerenijim temperaturama (60–100° C). Korišćenjem ove metode moguće je proizvesti preciznu strukturu rešetke, ako se temperatura precizno kontroliše i optimizuje sa parametrima brzine tokom generacije filamenta sa potrebnom tačnošću. Novije generacije FDM sistema koriste zagrejan rezervoar za ekstruziju polimernih peleta umesto vlakana. Skafoldi dobijeni ovom tehnikom iz alginata i PCL implantirani u miševe pokazali su ubrzano formiranje hrskavice i kolagena četiri nedelje nakon implantacije [8].

Smanjenje veličine mlaznice i visine sloja povećava x-y i z rezoluciju, što dovodi do znatno sporije brzine ekstruzije. Teorijska rezolucija je ograničena preciznošću linerano pokretnih sistema (motori, zupčanici, zupčasti kaiševi i navojne šipke) i retencionim svojstvima ekstrudiranog materijala. Iako FDM tehnike omogućavaju postizanje visokog stepena pozicione preciznosti u X-Y ravni, njihovo značajno ograničenje je u nemogućnosti štampanja visećih ili nepodržanih delova jer ne postoji potporni materijal iz prethodnih slojeva. Zbog toga je očvršćavanje tokom hlađenja ili unakrsno povezivanje nakon ekstruzije neophodno za zadovoljavajuću podršku sledećim slojevima. Takođe, ovaj nedostatak se može rešiti uvođenjem filamenta materijala za podršku tokom procesa štampanja, obično pomoću dodatnog ekstrudera (Slika 3).

Nedavna poboljšanja reoloških svojstava hidrogelova omogućila su štampanje ovih materijala pomoću FDM metode. Na primer, Hong sa sar. su napravili hidrogel koji se može štampati koristeći mrežu PEG i alginata sa silikatnim nanopločicama [16]. Ovi gelovi su posedovali nultu viskoznost iznad 10 kPa•s, što omogućava da zadrže oblik nakon štampanja i da se stanjuju pri smicanju, što olakšava ekstruziju. Veličina i tačnost štampanog konstrukta hidrogela zavise od zapremine sadržane u špricu i reoloških osobina hidrogela. Viskoznost igra ključnu ulogu u tačnosti konstruisanja, jer materijali visokog viskoziteta poseduju strukturnu rigidnost koja je važna za po-

držku ekstrudiranih uzastopnih slojeva, jer se sekundarni korak ukrštanja tipično koristi za zaključavanje štampanog oblika i za poboljšanje mehaničkih osobina ovih konstrukcija.

Štampači zasnovani na ekstruziji obično koriste pneumatski pritisak ili klip pokrenut motorom za nanošenje materijala. Pneumatski sistemi pojednostavljuju kontrolu primenjene sile na ekstrudirani materijal. Sistem treba kalibrisati za svaki materijal sa podešavanjem veličine mlaznice, geometrije mlaznice (koničnost vrha, cilindričnost igle i dužina) i pritiska gasa.

Izgleda da je FDM jedna od najsvestranijih tehnika štampanja za kreiranje biomimičnih skafolda zahvaljujući svojoj sposobnosti da napravi višeslojne konstrukcije izgrađene od različitih materijala i štampa mekane biomaterijale poput hidrogelova (Slika 4). Skafoldi štampani ovom tehnikom mogu pokazati anizotropne mehaničke osobine koje mogu biti korisne za proizvodnju skafolda sa namernim poravnanjem vlakana, kao što su ligament ili tetiva.

Bioprinting

Bioprinting pripada tehnikama aditivne štampe (ili aditivne proizvodnje) za kreaciju skafoda na bazi ćelija [17]. Ove tehnike su dominantno namenjene za štampu ćelijama istovremeno sa materijalom, budući da imaju minimalan uticaj na vijabilnost i funkciju ćelija. Biološki materijali koišćeni za štampu bi trebalo da se podudaraju sa prirodnim okruženjem tkiva domaćina da bi podržali funkcionalnost ovih ćelija. Pritom, ćelije bi trebalo da budu u stanju da prevaziđu mehanički stres tokom procesa štampe i prežive njegove realno nefiziološke uslove [8].

Tehnike bioprintinga su klasifikovane u tri kategorije: mikroekstruzija, bioprinting uz pomoć lasera (LAB) i inkdžet bioprinting. Među njima, inkdžet bioprinting je tehnika koja najviše obećava u stvaranju složenih arhitektura, uspešno podražava nativna tkiva i organe. U inkdžet bioprintingu kapi „bio-mastila" se nanose na supstrat u obliku gela da formira polimernu strukturu, dok bioprinting mikroekstruzijom koristi mehanički ekstruder da nanese biomastilo. Bioprinting ekstruzija je korištan za veću gustinu ćelija, zbog lakše tehnologije izvođenja, ali je sporiji nego bioprinting kapljicama. LAB tehnika zahteva pikolitarsku (pL) rezoluciju, kroz koju se ćelije i tečni materijali mogu štampati. Ovaj metod štampe se brzo razvija i pokazuje se perspektivnim za proizvodnji tkivolikih struktura.

Ekstruziono bio-štampanje

Ekstruziono bio-štampanje je jedna od najekonomičnijih tehnika brzog prototipovanja (Slika 5) [8, 17]. Ekstruziono bio-štampanje tipično uključuje pritisak ili vijčano/klipno aktiviranje izlaska tečnosti koja sadrži ćelije i/ili biomaterijale. Trebalo bi da obezbedi istanjivanje i smicanjem omogući minimalni otpor pri protoku i brzu hemijsku ili fizičku unakrsnu vezu nakon ekstruzije, kako bi se podržali sukcesivni slojevi. Ova tehnika omogućava preciznu depoziciju materijala i izradu složenih struktura, uključujući i upotrebu više vrsta ćelija, omogućavajući na taj način ubrzani rast i formiranje novih tkiva. Poželjno je povećanje rezolucije štampanja i brzine štampanja. Pored toga, modifikacijom mehanike štampanja vreme štampanja se može smanjiti i može se postići koekstruzija više materijala. Glavni nedostatak je relativno dugo vreme izrade kako bi se postigla visoka rezolucija u kompleksnim strukturama. Ova metoda omogućava uspešnu izradu klinički relevantnih skafolda za tkivno inženjerstvo, jer je idealno prilagođena biološkim materijalima zahvaljujući svojoj sposobnosti da deponuje više materijala sa širokim opsegom svojstava. Skafoldi dobijeni ekstruzionim bio-štampanjem su obično mekani, zbog visokog sadržaja vode, što ih čini ograničenim za primenu samo kod mekih tkiva.

Laserski potpomognuto bio-štampanje

LAB, ili biološko lasersko štampanje, grupa je laserskih tehnika koje koriste lasersku energiju kako bi olakšale densifikaciju materijala skafolda (Slika 6) [8, 17]. Jedan tip LAB-a podrazumeva upotrebu laserskog pulsa (LDV) za lokalno zagrevanje slajda sa slojem koji apsorbuje energiju i rastvorom ćelija. Laserski puls indukuje sublimaciju ili isparavanje materijala, proterujući rastvor ćelija na suprotnoj strani i precizno ih nanoseći na podlogu. Ova metoda uključuje laserski indukovani transfer i lasersko isparavanje pomoću matrice, koji se mogu koristiti za depoziciju fibroblasta, keratinocita, humanih mezenhimalnih matičnih ćelija, različitih kancerskih ćelija i biopolimera.

Kako laserske tehnike omogućavaju visoku preciznost, ova metoda je pogodna za bio-štampanje najmanjih detalja nativnih tkiva i organa. Ova tehnika pruža mogućnost direktnog štampanja ćelija, ali uz nekoliko ograničenja, kao što su štetan efekat na ćelijsko preživljavanje i njihovo dugoročno ponašanje.

Inkdžet bio-štampanje

Inkdžet bio-štampanje omogućava preciznu depoziciju ćelija i biomaterijala, koristeći neke pogodnosti 2D inkdžet štampanja kako bi nastali 3D skafoldi [8, 18]. U ovom postupku ograničena zapremina tečnosti pada u precizan kalup kreiran pomoću odgovarajućeg softvera. Jedna od najvažnijih prednosti ove tehnike je brzina kojom se mogu proizvoditi skafoldi sa vrlo kompleksnom 3D arhitekturom. Ova velika brzina ograničava broj polimernih materijala koji se mogu koristiti za bio-štampanje, jer njihovo vreme geliranja mora biti veće ili jednako vremenu depozicije kapi.

Inkdžet bio-štampači se mogu prilagoditi za štampanje materijala sa povećanom rezolucijom i brzinom. Oni koriste termičku ili piezoelektričnu energiju da deponuju kapljice rastvora u željene kalupe i sastoje se od jedne ili više komora sa mastilom sa više mlaznica i sa odgovarajućim piezoelektričnim ili grejnim komponentama. Kratak impuls struje se koristi za aktiviranje komponente i izbacivanje kapljice mastila. U termičkim bio-štampačima često se javlja jak lokalni porast temperature, izazivajući stvaranje i pucanje mehurića, što dovodi do izbacivanja kapljica mastila na podlogu. U piezoelektričnoj inkdžet štampi, piezo-kristali indukuju povećanje pritiska, što dalje utiče na izbacivanje kapljice. Depozicija od mlaznice na površinu štampe se dešava kada električni naboj izaziva vibracije u kristalima, a vibracije se šire na površinu za štampanje. Pokazano je da toplotna i mehanička opterećenja pri termičkom bio-štampanju uzrokuju smanjenje vijabilnosti ćelija. Neki istraživači su koristili ovu metodu za štampanje retinalnih ganglija i glia ćelija izolovanih iz centralnog nervnog sistema odraslih bez negativnog uticaja na preživljavanje ćelija, dok su neki od njih uspeli da koriste termo osetljive gelove modifikovanjem kertridža komercijalno dostupnog inkdžet štampača za kreiranje višeslojnih skafolda [8].

Glavni nedostatak inkdžet štampanja je zahtev da biološki agensi budu u tečnom stanju, kako bi se omogućila depozicija. Odložene kapljice zatim očvršćavaju u potrebnu geometriju, preko unakrsnog povezivanja na osnovu fizičkih, hemijskih, pH ili ultraljubičastih metoda. Zbog hemijskog unakrsnog povezivanja, mnogi prirodni materijali često menjaju svoje hemijske osobine. Osim toga, neki mehanizmi unakrsnog povezivanja indukuju smanjenje ćelijske vijabilnosti i funkcionalnosti (Slika 7).

Iako inkdžet bioprinting omogućava enkapsulaciju živih ćelija, njihova koncentracija mora da bude relativno niska da bi se omogućilo formiranje kapljice i sprečilo začepljenje dizne. Uprkos brojnim manama, ovaj metod ima veliki potencijal zbog svoje niske cene, visoke rezolucije i visoke kompatibilnosti sa mnogim biomaterijalima. Ovakvi štampači omogućavaju tačnu depoziciju finih kapljica precizne zapremine da bi stvorili skafolde visoke rezolucije sa netaknutim ćeijama. Veličina kapljica može biti podešavana između 1 i 300 pL sa brzinama depozicije od 1 do 10.000 kapljica u sekundi. Prema tome, ovaj metod omogućava stvaranje skafolda sa preciznošću od 100 μm, što je veoma obećavajuće za stvaranje složenih skafolda. Iako zbog tipičnih svojstava gel-mastila ne može proizvesti veoma visoke strukture, vrlo je pogodan za štampu kompleksnih tkivnih struktura sa velikom preciznošću zbog svoje sposobnosti da štampa više struktura i tipova ćelija.

OČEKIVANA SUDBINA OVIH METODA U BUDUĆNOSTI

Adaptacija postojećih metoda 3D štampanja za biološke primene je od ogromnog značaja za buduću proizvodnju tkivnih graftova i veštačkih organa. Pored inženjerstva tkiva, 3D štampanje se takođe koristi u oblasti isporuke lekova, analizi hemijskih i bioloških agenasa i organa na čipu uređaja [19].

Uprkos svom velikom potencijalu u regenerativnim strategijama, glavni izazovi se odnose na poboljšanje rezolucije, povećanje brzine i štampanja koje omogućava preživljavanje ćelija [18]. Tekući napori u unapređenju rezolucije štampanja u litografiji podrazumevaju razvoj metoda kao što su litografija elektronskim snopom i multifotonska apsorpciona polimerizacija, jer su ove metode pogodne za izradu skafolda sa veoma preciznom veličinom detalja, reda samo nekoliko desetina nanometara [20].

Materijali koji se koriste za 3D bio-štampanje moraju zadovoljiti sledeće kriterijume: treba da budu biokompatibilni, da podrže rast i diferencijaciju ćelija i da zadrže oblik dovoljno dugo da bi se očuvao integritet skafolda dok se ne završi proces očvršćavanja unutar geometrije skafolda. Najviše korišćeni materijali za ove svrhe su kolagen, želatin, hijaluronska kiselina, alginate, modifikovani kopolimeri i fotopolimerizujući makromeri [21].

Za dizajniranje kompleksnih skafolda koji imitiraju tkivo neophodna su dodatna istraživanja za tačno mapiranje kompleksnih tkiva da bi mogli da se naprave reproducibilni skafoldi sa zahtevanim strukturnim i biološkim osobinama. Jedan od glavnih izazova u budućem 3D štampanju je direktno bio-štampanje ili štampanje ćelija i biomaterijala direktno na pacijentu ili u pacijenta. Neke nedavne studije su pokazale mogućnost bio-štampanja direktno na rane ili opekotine [22]. Dalja unapređenja brzine štampanja i rezolucije su neophodna za *in situ* štampanje, koje će unaprediti regeneraciju tkiva i redukovati vreme oporavka pacijenta.

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The evolution of articulators – part I

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SUMMARY

One of the objectives of prosthetic dentistry is to restore teeth in harmony with temporo-mandibular joints. Articulator is a device that allows an operator to fabricate a restoration that will be physiologically and psychologically successful. But how much do we really know about the origin of articulators? First articulator designs attempted to duplicate anatomic relationships and functional movements. More sophisticated articulators evolved as more new knowledge about anatomy, mandibular movements and mechanical principles were obtained.

The aim of this article was to describe the origin, history and evolution of articulators through the years.

For this article, an electronic search was performed across three databases (Science Direct, PubMed and Google Scholar) for relevant citations. Keywords such as articulators, history, early articulators were used alone and in combination for the search. The option of "related articles" was also utilized. Finally, search was performed on the review articles and the most relevant papers were selected.

Keywords: articulators; instruments; cast; temporo-mandibular joints; hinge

INTRODUCTION

Articulators are mechanical instruments that represent maxilla, mandible and temporomandibular joints (TMJs). Their main task is to provide a frame where it is possible to relate, in the three planes of space, maxillary cast with mandibular cast relative to the hinge axis of the patient and of the instrument [1].

An articulator can be defined as following: "It is a mechanical instrument that represents temporomandibular joints and jaws, to which maxillary and mandibular casts may be attached to simulate some or all mandibular movements" [2].

Early articulators were based on individual theories of occlusion. However, normal variations in mandibular movement between patients, and even variable movement of the joints in one patient required to design adjustable articulators. The challenge for the dentist is to choose an articulator that is suitable for specific purpose. This requires an understanding of the instrument, as well as a grasp of the treatment objectives for the given patient [3].

Very little is known about the origin of dental articulators. There are two early documented facts: 1) Phillip Pfaff was the first to describe wax impression procedure and a method of making plaster casts; and 2) Jean Baptiste Gariot was the first one to describe a method for mounting casts and preserving their relationship with plaster index ("plaster articulator"). However, it must be recognized that because Gariot never claimed the procedure as an innovation, it was not associated to him. Even though "plaster articulators" were the first to be used for preserving the relationships of casts, sometime before 1840, mechanical hinge articulators have become a new and widely used device for this purpose [4]. More sophisticated articulating instruments evolved as more knoledge about anatomy, mandibular movement, and mechanical principles was obtained.

Many articles in dental literature pertaining to articulators deal with the theory and development of articulating instruments, directions for use, explanations of deficiencies and methods for overcoming them, detailed mechanical interpretations of the instruments, and various systems of classification [5].

The aim of this article was to describe the history of articulators and how they have evolved in time.

MATERIAL AND METHOD

An electronic search was performed across three databases (Science Direct, PubMed and Google Scholar) for relevant citations. Keywords such as articulators, history, early articulators were used alone and in combination for the search. The option of "related articles" was also utilized. Finally, a search was performed on the review articles and the most relevant papers pertaining to the history of articulators were selected.

Over the years some articulators have been modified, some have not been accepted by the profession and many are no longer in use [6]. Our article describes the history of articulators, beginning with the 18th century, when the evidence of the first articulator appeared.

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Plaster Articulator

Phillip Pfaff, dentist to the court of Frederick the Great, King of Prussia, first described his method of preserving the relationship of the casts, the plaster articulator in 1756 (Figure 1) [4]. It consisted of a plaster extension on the distal portion of the mandibular cast, which was grooved to serve as a guide for plaster extension of the maxillary cast. This was the first articulator, commonly known as 'slab articulator' [5].

Barn Door Hinge

In 1805, Jean Baptiste Gariot described his method of making plaster casts and extending them posteriorly to provide an indexing mechanism for preserving the relationship of the casts [4]. The barn door hinge (Figure 2), designed by J.B.Gariot, had an anterior vertical stop. It accepted centric relation record and reproduced this position reliably [5].

Hovarth and Ladmore Articulators

The first published reference to the earliest mechanical hinge articulators is probably Fairhurst's discussion of Hovarth's articulator and Ladmore's articulator in the 1830s. Fairhurst described these and other early examples as instruments consisting of two wooden or metal blocks or slabs hinged together with a simple hinge. These articulators and others of those day must certainly have resembled what is now commonly referred to as the "barn door hinge" [7].

Thomas W. Evan's Articulator

One of the earliest mechanical hinge articulators illustrated and discussed in some detail in the literature is attributed to Thomas W Evans. In his textbook, Chapin A Harris described the T W Evans articulator as a "very simple instrument by means of which the extension of the plaster back of the plates and wax is rendered unnecessary." He emphasized that the most important feature was that vertical dimension could be preserved or altered as required. It is not known if Thomas Evans patented his Articulator. According to House, the records of the US patent office before 1870 may be incomplete. In that year, the Commissioner of Patents completely reorganized the system of keeping records and issuing patent letters. Furthermore, sometime between 1840, when the first patents were issued, and 1870, a fire destroyed many of the original patent records [7].

The First US Articulator Patent – Cameron's Articulator

The first US patent for articulator was issued to James Cameron on April 30, 1840. Cameron's articulator was unique in its departure in design from other hinge-type devices (Figure 3). There was inclusion of the anterior-posterior and vertical adjustment features [7].

The Second US Articulator Patent – Even's Articulator

The second articulator to be patented was that of Daniel T. Even (Figure 4). He first attempted to record mandibular movement and recognised the forward and lateral movement of the mandible. He named it the "Dentist's Guide". It was met with little appreciation, and consequently, disappeared from the scene. It was unsuccessful because it was inconvenient and difficult to use.[8]

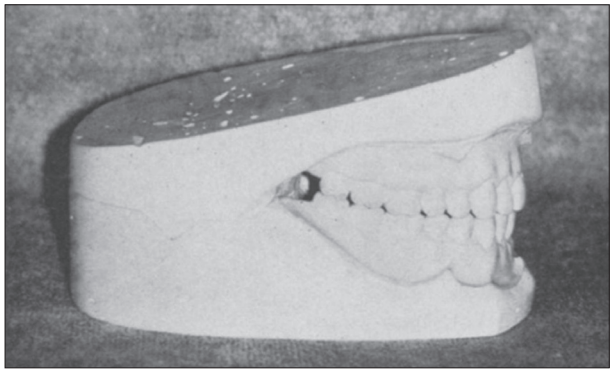

Figure 1. Plaster Articulator [5]
Slika 1. Gipsani artikulator [5]

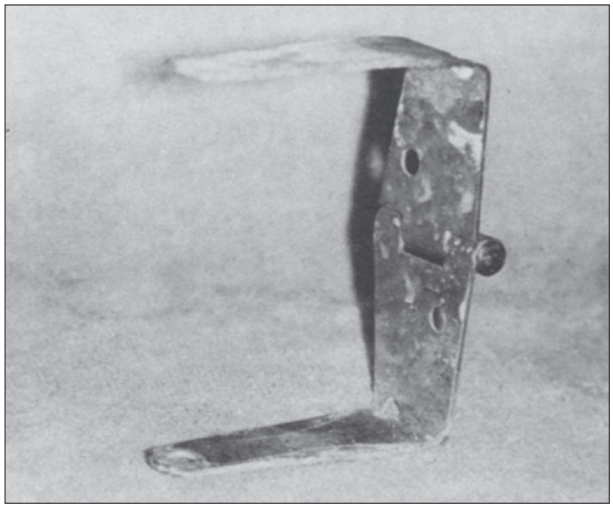

Figure 2. Barn Door Hinge [5]
Slika 2. Osovina šarnir vrata [5]

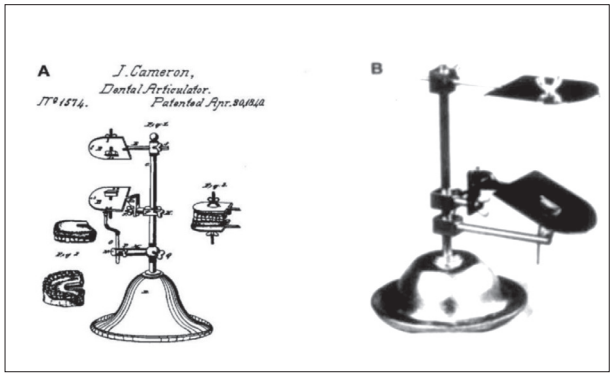

Figure 3. Cameron's Articulator [7]
Slika 3. Kameronov artikulator [7]

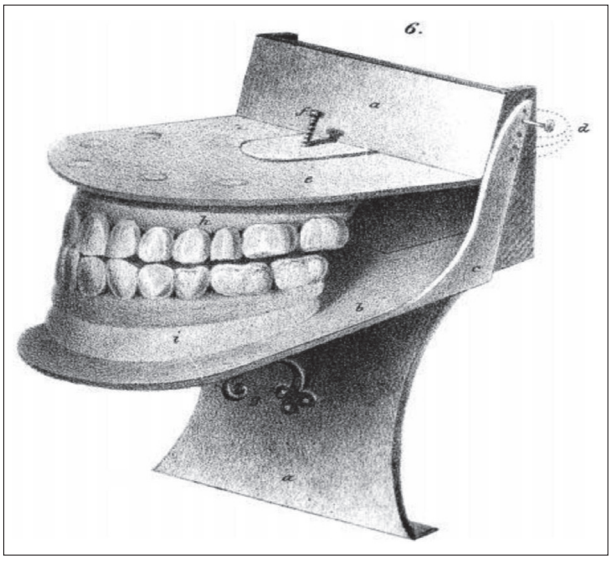

Figure 4. Even's Articulator [8]
Slika 4. Evenov artikulator [8]

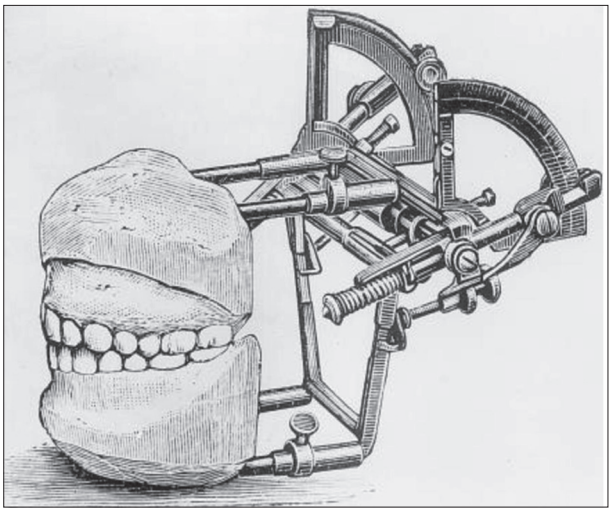

Figure 5. Walker Articulator [9]
Slika 5. Vokerov artikulator [9]

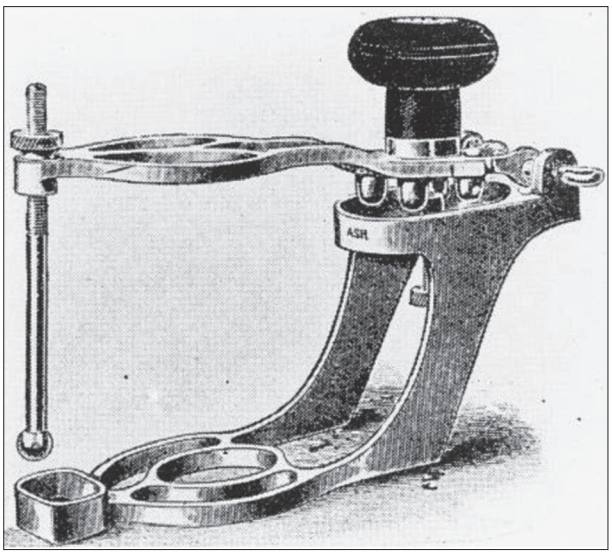

Figure 6. Luce Articulator [10]
Slika 6. Artikulator Luce [10]

"Fixed Condylar Guide Articulators" And The Next Advancement

The original Evens Articulator, with the horizontal condylar path feature, can be classified generally as a "Fixed Condylar Guide" instrument, and is the earliest of this type. Two other early patented examples were the Starr (1868), and the Antes- Lewis (1895, 1900). The most well-known articulator in this category was the Bonwill (1858). All of these articulators received some acceptance by the profession. Indeed, the Bonwill was popular for many years. But none represented a further innovation in principle. It was not until almost 50 years after the Evens was patented that Richmond S. Hayes would incorporate the next important advancement into an articulator, the downward and forward condylar path, when he issued a patent for his "fixed condylar guide" instrument in 1889 [8].

The First "Adjustable Condylar Guide" Articulator

The next important breakthrough in articulator design occurred in 1896, when William E. Walker of Pass Christian, MS obtained 2 patents for articulators with adjustable condylar guides (Figure 5). The earliest version of his articulator, constructed in about 1895, had fixed condylar guides. Although the second model, featured adjustable condylar controls, they could not be set individually. The third model of the Walker articulator was the first to include individually adjustable condylar guides as well as adjustable rotation centers for lateral movement. Walker patented these last 2 models sequentially, calling them "Walker's Physiological Articulators". To measure the path of each condyle individually Walker designed an apparatus that he called "*facial clinometer*" and procedure for its use. Because it was designed to determine the angle of the condylar paths on the face, it can be considered first *extraoral* method for recording mandibular movement, although the paths were not actually scribed. Facial clinometer was never patented [9].

The Appearance And Early Use Of The Incisal-Pin And Guide [10]

As early as the 1840s, some form of "*vertical stop*" was common component of mechanical articulators. Clearly, some early inventors recognized the importance of preserving vertical relationship of the casts in the articulator and provided this feature.

The First Articulators Patented With An Incisal- Pin And Guide Assembly

C.E. Luce, of Stuttgart, Germany, received the first patent for an articulator with an incisal-pin and guide assembly on November 28, 1911. Luce was also one of the first investigators to describe downward and forward movements of condyles (1889). Luce's articulator was the first "scribing" type; that is, it had posterior and incisal path controls that were functionally generated (Figure 6).

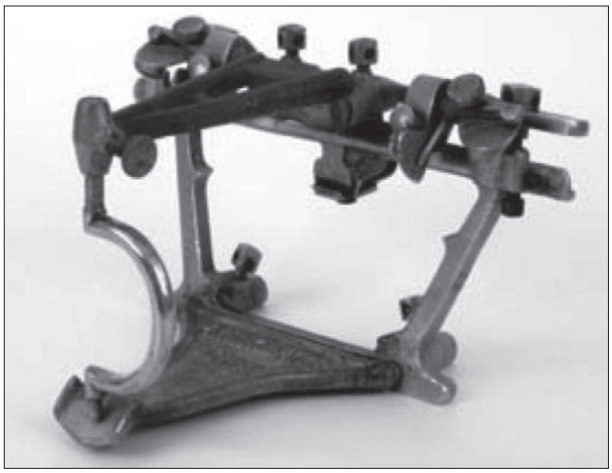

Figure 7. Gysi's Adaptable Instrument [10]
Slika 7. Gysijev adaptabilni instrument [10]

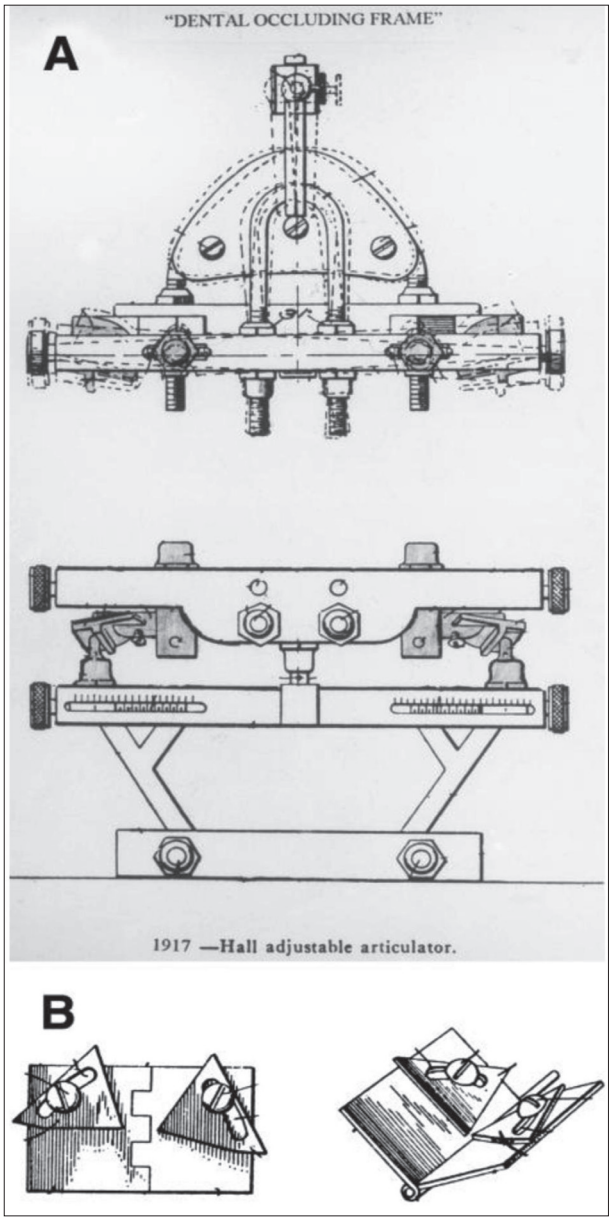

Figure 8. Hall's Occluding Instrument [10]
Slika 8. Holov okludirajući instrument [10]

The second patent issued for an articulator with this feature was received by Ernest Eltner of Basel, Switzerland in July 1912. Eltner's articulator featured horizontal incisal guide table with adjustable posterior vertical flange that limited protrusive movements. The incisal pin had a chevron-shaped blade tip.

Alfred Gysi received a patent for his "*Adaptable*" articulator in October 1912 (Figure 7). This was Gysi's first US articulator patent. Although the "*Adaptable*" was highly advanced for the time, it had only fixed, 45-degree incisal guide table. Nevertheless, the "*Adaptable*" was considered too complicated for the average practitioner and did not achieve commercial success. Gysi used the exact incisal guide design in his "*Simplex*" articulator, an "average value" version of the "*Adaptable*" articulator.

Ray W. Burch of Hart, MI received the fourth patent issued for an articulator with incisal pin, in December 1913. Burch was the first to employ the innovation of converting maxillary and mandibular facebows into an articulator. The facebow devices were connected by condylar slot assemblies and were adjusted while being worn by the patient. The incisal pin was used simply to maintain the anterior vertical position.

After the Burch articulator, George B. Snow patented the *"Acme"* articulator in November 1915, which was Snow's third articulator patent and the first with incisal-pin and guide. In the earliest model, the protrusive angle of the incisal table was adjusted by heating the solder between the components identified by the numbers *4* and *8*, and moving the table up or down. Later models featured interchangeable tables, and finally, an adjustable table with a setscrew.

David M. Shaw of Eltham, England, patented an articulator in February 1916. This device has been largely overlooked, but deserves mentioning due to the notable design of the incisal pin assembly, apparently intended to affect protrusive and lateral motion with the curved incisal chevron-shaped tip functioning on the curved wire "guide."

In November 1916, Rupert E. Hall received his first patent for an articulator, a device that has been unceremoniously nicknamed "*Alligator*". The "*Alligator*" was the first articulator to include an incisal guide table with adjustable lateral wings. On Hall's second articulator (March 1917), a revised version of the "*Alligator*," the incisal pin was designed to function within a 45-degree cup.

Hall's "Dental Occluding Frame": The First Patented Gothic Arch Incisal Guide Table

Rupert Hall's third articulator patent, issued for "*Dental Occluding Frame*" (April 1917), was groundbreaking (Figure 8). It was an arcon instrument that featured adjustable, curved condylar paths, including settings for the Balkwill-Bennett angle, and a mechanism for adjusting the intercondylar distance. It also included horizontal incisal table with adjustable lateral wings, as well as new feature - a triangular guiding edge on each lateral wing that could be set to follow "gothic arch" tracing.

Figure 9. Maxillomandibular Instrument [5]
Slika 9. Maksilomandibularni instrument [5]

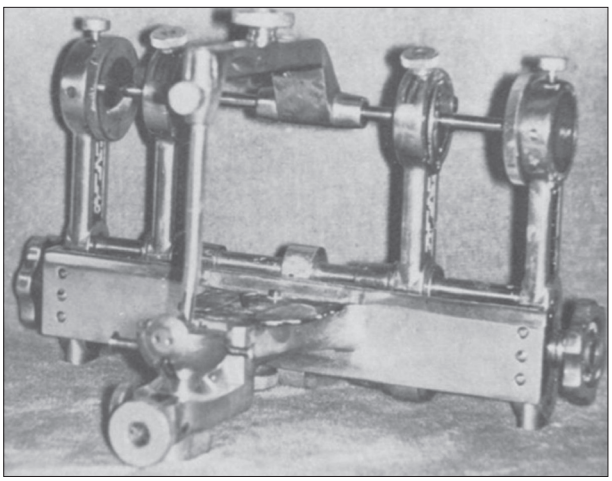

Figure 10. Hanau Model M Kinescope [5]
Slika 10. Kinetoskop Hanau Model M [5]

Maxillomandibular Instrument

It was designed by George Monson in 1918, and was based on the spherical theory. According to this theory, which evolved from the concepts of Monson and a German anatomist Graf von Spee, the mandibular teeth move over the maxillary teeth as over the surface of a sphere. The average radius of the sphere is 4 inches, but the Monson instrument has set screws that can vary the instrument's radius (Figure 9) [5].

Stephen Articulator

Stephen articulator, developed in 1921, is similar in design to the Gariot hinge articulator from 1805, except that it has a fixed condylar inclination and allows for an arbitrary lateral movement. A posterior set screw holds the upper and lower members of the articulator at fixed vertical dimension [5].

The Hanau Model C Articulator And The Hanau Model M Kinoscope

Rudolph L. Hanau, an engineer, was influenced by Dr. Rupert E. Hall to study the design of articulators. Early in 1921 he developed a research model called the Hanau Model C articulator. In 1923, he developed another research instrument, the Hanau Model M Kinoscope articulator (Figure 10). It has double condylar posts on each side. The inner posts serve two purposes- (1) they act as the horizontal condylar guides and (2) they are variable rotation centers when the posts are moved inward or outward. The Bennett angle is adjusted by rotating an eccentric cone located on the outer posts against the intercondylar axis [5].

Homer Relator

Homer Relator was introduced in 1923 by Joseph Homer. It was based on a principle that plastic material rather than mechanical guides is used to preserve articulator positions. Three cups in the lower member filled with plastic material (usually modeling compound), capture the record and guide the tripoded upper member into the recorded positions. The same principle has been used later in the Irish duplifunctional and TMJ instruments [5].

Wadsworth Articulator

Wadsworth believed in Monson's spherical theory but he could not accept bilateral condylar symmetry. In the Wadsworth articulator, developed in 1924, the casts were mounted with a facebow and the Wadsworth T-attachment determined the third point of reference. A divider was used to measure the distance from the median incisal point to the condyle center on one side. This arc length was described first from the condyle and then from the median incisal point to the flag located on the instrument's upper member. The intersection of these arcs was located on the rotational center for the measured side. The center was used to design a spherical plane of occlusion. It had an adjustable intercondylar distance as well. This measurement was determined by using the distance between the facebow condyle pointers minus 0.75 inch skin-condyle distance on each side. The condylar paths of the instrument were slightly curved [5].

The Hanau Model H110

The Hanau Model H110 was introduced by Hanau in 1926 and was designed primarily for complete denture and to encompass mechanical averages of many previous concepts. It has individual condylar guidance adjustments in both sagittal and horinzontal planes [5]. Rather than using lateral positional records, the lateral setting was calculated using the formula, given at the base of the articulator:

$$L = H/8 + 12,$$ where H = Horizontal condylar angle.

The Hanau Model H110 Modified

It was designed in 1927, and introduced the incisal guide table. The original incisal guide cup with its fixed curvature could be moved only as a unit, and it did not have calibrations for resetting. The improved table appeared on Hanau articulators from 1927 to 1972 and allowed for adjustments in three dimensions through considerable range [5].

The Hagman Balancer

Developed in 1920s by H. C. Hagman, the Hagman Balancer opens and closes on a hinge that is in the center of the upright support but requires no facebow or interocclusal records for mounting. A centering device transfers maxillary and mandibular impressions form the patient to the articulator in one step. It was also based on spherical theory of occlusion. Mandibular teeth are constructed to the curve of Spee using a balanced occlusal guide, and maxillary teeth are constructed to fit with mandibular teeth [5].

Phillips Student Articulator

The Phillips Student articulator (Model C), or the Pantographic articulator, was developed by George P. Phillips in 1926 (Figure 11). This articulator is classified as fully adaptable, as its developer claimed that it would follow any graphic record. The Phillips graphic recorder was designed to trace in one step the Gothic arch (needlepoint) tracing and the inclinations of glenoid fossa. The articulator could reproduce mechanically the movements of the graphic recorder through the use of two vertical pins that follow horizontal inclination of glenoid fossa, and two horizontal pins that retraced needle point tracing [5].

Stanbery Tripod Instrument

Developed in 1929 by C. J. Stansbery, this articulator was designed without a hinge to facilitate the reproduction of any positional relationship. There is no mechanical equivalent or representation of condyles. The articulator reproduces positions, not movements. Interocclusal positional records of centric, protrusive, right lateral and left lateral positions are used to set three individual turrets and slots of the tripod, with the slots forming straight line to the centric position [5].

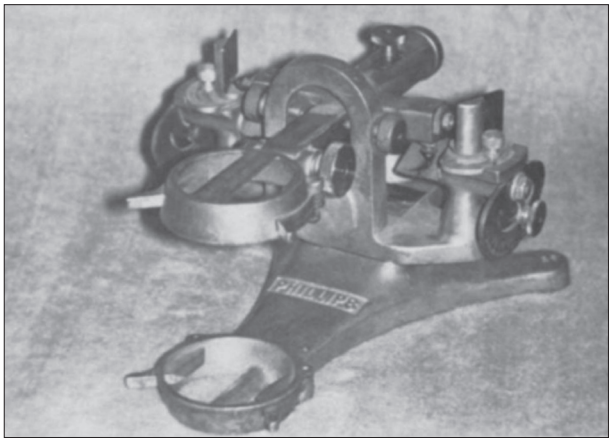

Figure 11. Phillip's Student Articulator [5]
Slika 11. Filips Studentov artikulator [5]

House Articulator

The House articulator was developed by M. M. House in early 1930s. The Needle-House intraoral chew-in or other positional records were used to set the House articulator. The intercondylar centers of rotation varied without moving the lateral posts that support the condylar elements, with the help of hooks that could slide along the intercondylar bar. The lateral condylar guidance was controlled by the Bennett guide, attached lateral to the condylar guide slot. The incisal guide table could control horizontal and vertical movement. Lateral plates in the guide table created a mechanical function as a curved incisal pin [5].

Precision Coordinator

The Precision Coordinator was developed by W. H. Terrell in the early 1930s. It is an arcon type of articulator that has curvilinear condylar guides. Twin parabolic cams control vertical and horizontal anterior guidance. The incisal pin is curved to allow for changes in the vertical dimension. There is also freedom of movement in centric relation [5].

The Hanau Crown And Bridge Articulator

The Hanau Crown and Bridge articulator 29-0 was manufactured by the Hanau Engineering Co from 1934 to 1971. It is a small articulator. A posterior pin-and-cam guidance mechanism can be set to simulate working and balancing side excursions of 15 degrees. The mechanism can be set to L for restorations in patient's left quadrant, R for right quadrant, or Anterior restorations or for equalizing right and left excursions. Its protrusive movements were up to 30 degrees. No facebow was needed [5].

The Philips Occlusoscope

The Philips Occlusoscope articulator was developed by George P. Phillips in 1938. The maxillary cast on the The Philips Occlusoscope articulator was mounted with the use of a facebow. The articulator could be adjusted by either intraoral or extraoral records. The lower member had two adjustable units that represents the two tempormandibular joints. Within each adjustable unit, there was a circular disc that could be tilted anteroposteriorly and laterally. It did not have an adjustable incisal guide. The incisal pin rested on a flat plane because Philips believed that an incisal guide pin serves only to prevent closure and should not serve as a third tempomandibular joint [5].

CONCLUSION

Articulators are instruments that attempt to reproduce the range of movement of the jaw. The first instrument designs attempted to duplicate anatomic relationships or

reproduce functional movements of the anatomy. More sophisticated articulating instruments evolved as more was learned about anatomy, mandibular movements, and mechanical principles. However, the objective was always same: to produce or reproduce occlusal relationships extraorally. No matter how simple or complicated an articulator may be, if the operator does not use it properly or if it does not have the features for the basic purpose for which is used, the results will be disappointing [3]. A history of articulators since 1940 will be published in the next part of the article.

REFERENCES

1. Winkler S. Essentials of complete denture prosthodontics, 2nd Indian edn. AITBS Publishers. 2009; 142-3.
2. Glossary Of Prosthodontic Terms. J Prosthet Dent. 2005; 94:10-92.
3. Zarb GA, Bolender CL. Prosthodontic Treatment For The Edentulous Patient, 12th edn. St Louis: C.V. Mosby; 2004; 291-2.
4. Starcke Edgar N. The history of articulators: A perspective on the early years, Part I. J Prosthodont. 1999; 8:209-11.
5. Mitchell DL, Wilkie ND. Articulators through the years. Part I. J Prosthet Dent. 1978; 39:330-8.
6. Mitchell DL, Wilkie ND. Articulators through the years. Part II. J Prosthet Dent. 1978; 39:451-8.
7. Starcke Edgar N. The history of articulators: A perspective on the early years, Part II. J Prosthodont. 1999; 8:277-80.
8. Starcke Edgar N**.** The history of articulators: Early attempts to reproduce mandibular movement. J Prosthodont. 2000; 9:51-6.
9. Starcke Edgar N. The history of articulators: Early attempts to reproduce mandibular movement, Part III. J Prosthodont. 2000; 9:217-22.
10. Starcke Edgar N. The history of articulators: The appearance and early use of the incisal pin and guide. J Prosthodont. 2001; 10:52-60.

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Istorija artikulatora – prvi deo

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KRATAK SADRŽAJ

Jedan od ciljeva stomatološke protetike je da protetske nadoknade zuba uradi u skladu sa temporomandibularnim zglobovima. Artikulator je uređaj koji omogućava stomatologu da proizvede protetsku nadoknadu koja će biti fiziološki i psihološki uspešna. Ali koliko znamo o poreklu artikulatora? Prvi dizajni artikulatora imali su za cilj da dupliraju anatomske odnose i funkcionalne pokrete. Sofisticiraniji artikulatori nastali su zahvaljujući novim saznanjima o anatomiji, pokretima donje vilice i mehaničkim principima. Cilj ovog rada bio je da opiše poreklo, istoriju i evoluciju artikulatora.

Izvršena je elektronska pretraga u tri baze podataka (Science Direct, PubMed i Google Scholar) za relevantne citate. Ključne reči kao što su artikulatori, istorija i rani artikulatori su korišćene same ili u kombinaciji. Korišćena je i opcija „povezanih članaka". Na kraju, izvršena je pretraga revijalnih radova i odabrani su najrelevantniji radovi.

Ključne reči: artikulatori; instrumenti; gipsani modeli; temporomandibularni zglobovi; osovina

UVOD

Artikulatori su mehanički instrumenti koji simuliraju gornju i donju vilicu i temporomandibularne zglobove (TMZ). Njihov glavni zadatak je da obezbede okvir u kome je moguće, u tri ravni, upostaviti odnos gipsanih modela gornje i donje vilice u odnosu na osu zgloba pacijenta i instrumenta [1]. Artikulator se može definisati na sledeći način: „To je mehanički instrument koji predstavlja temporomandibularne zglobove i vilice, na koji se mogu pričvrstiti gornji i donji gipsani modeli vilice i koji simuliraju neke ili sve pokrete donje vilice" [2]. Rani artikulatori su bazirani na individualnim teorijama okluzije. Međutim, normalne varijacije kretnji donje vilice kod različitih pacijenata, pa čak i varijabilno kretanje zglobova kod jednog pacijenta, zahtevalo je dizajniranje prilagodljivih artikulatora. Izazov za stomatologa je izbor artikulatora koji je pogodan za specifičnu svrhu. Ovo zahteva razumevanje instrumenta, kao i razumevanje ciljeva tretmana datog pacijenta [3].

Veoma malo se zna o poreklu artikulatora. Postoje dve ranije dokumentovane činjenice: 1) Phillip Pfaff je prvi koji je opisao postupak uzimanja zubnih otisaka u vosku i izradu gipsanih modela i 2) Jean Baptiste Gariot je bio prvi koji je opisao metod za montažu gipsanih odela i očuvanje njihovog odnosa pomoću gipsanog indeksa („gipsani artikulator"). Međutim, s obzirom na to da Gariot nikada nije patentirao svoj postupak, on se i ne vezuje za njega. Iako su „gipsani artikulatori" bili prvi koji su se koristili za očuvanje odnosa gipsanih modela, negde pre 1840. godine, artikulatori sa mehaničkim osovinama su postali novi i široko upotrebljivani aparati za tu svrhu [4]. Sofisticiraniji artikulatori razvijeni su zahvaljujući novim saznanjima o anatomiji, pokretima donje vilice i mehaničkim principima.

Mnogi članci u stomatološkoj literaturi koji se odnose na artikulatore bave se teorijom i razvojem artikulacionih instrumenata, uputstvima za upotrebu, objašnjenjima nedostataka i metoda za njihovo prevazilaženje, detaljnim mehaničkim interpretacijama instrumenata i različitih sistema klasifikacije [5].

Cilj našeg rada bio je opisati istoriju artikulatora i njihov razvoj i usavršavanje.

MATERIJAL I METOD

Elektronska pretraga obavljena je kroz tri baze podataka (Science Direct, PubMed i Google Scholar) za relevantne citate. Ključne reči kao što su artikulatori, istorija, rani artikulatori korišćene su same ili u kombinaciji za pretraživanje. Korišćena je i opcija „povezanih članaka". Konačno, izvršena je pretraga revijalnih radova i izabrani su najvažniji citati koji se odnose na istoriju artikulatora.

Tokom godina su neki artikulatori modifikovani, neki od njih nisu prihvaćeni od strane profesije i mnogi se više ne koriste [6]. Naš rad opisuje istoriju artikulatora sve od 18. veka, kada su se pojavili dokazi prvog artikulatora.

Gips Artikulator

Phillip Pfaff, stomatolog suda Fridriha Velikog, kralja Pruske, prvi opisao svoj metod očuvanja odnosa gipsanih modela vilica pomoću gipsanih artikulatora 1756. god. (Slika 1) [4]. On se sastojao od gipsanog proširenja na zadnjem delu gipsanog modela donje vilice, koji je izgledao kao žleb i služio kao vodič za gipsano proširenje modela gornje vilice. Ovo je bio prvi artikulator, poznat kao „pločani artikulator" [5].

Osovina šarnir vrata (Barn Door Hinge)

Godine 1805. Jean Baptiste Gariot opisao je svoj način izrade gipsanih modela i njihovo proširenje na zadnjoj strani, kako bi se napravio mehanizam za očuvanje odnosa gornjeg i donjeg gipsanog modela [4]. Artikulator Barn Door Hinge (Slika 2), koji je dizajnirao J. B. Gariot, imao je prednji vertikalni stoper. Prihvatao je centralnu relaciju vilica i pouzdano je reprodukovao [5].

Artikulatori Hovarth i Ladmore

Prva objavljena referenca najstarijih artikulatora sa mehaničkom osovinom je verovatno Ferherstova diskusija o Hovartovom i Ladmorovom artikulatoru 1830-ih godina. Ferherst opisuje ove i druge rane primere kao instrumente koji se sastoje od dva drvena ili metalna bloka ili ploče koji su povezani

zajedno jednostavnom šarkom. Ovi artikulatori, kao i drugi iz tog vremena, sigurno su ličili na ono što se danas obično naziva „okludatorom“ [7].

Tomas Evanov artikulator

Jedan od najstarijih mehaničkih artikulatora osovine ilustrovan je i detaljno opisan u literaturi Tomasa V. Evana. U svom udžbeniku Čejpin A. Haris opisao je Tomas Evanov artikulator kao „vrlo jednostavan instrument“, kao i da je zadnje proširenje gipsanog modela i voska nepotrebno. Naglasio je da je najvažnija karakteristika bila da se vertikalna dimenzija može sačuvati ili izmeniti po potrebi. Nije poznato da li je Tomas Evan patentirao svoj artikulator. Prema Hausu, evidencija američke patentne kancelarije pre 1870. godine može biti nepotpuna. U toj godini poverenik patenata potpuno je reorganizovao sistem vođenja evidencije i izdavanja patenata. Štaviše, negde između 1840. godine, kada su prvi patenti izdati, i 1870. godine, vatra je uništila mnoge originalne zapise patenata [7].

Prvi američki patent artikulatora – Kameronov artikulator

Prvi američki patent za artikulator dobio je Džejms Kameron 30. aprila 1840. Kameronov artikulator je bio jedinstven u dizajnu u poređenju sa drugim artikulatorima tipa osovine (Slika 3). Imao je prednje i zadnje funkcije vertikalnog podešavanja [7].

Drugi američki patent artikulatora – Evenov artikulator

Danijel T. Even je patentirao drugi artikulator (Slika 4). To je prvi artikulator koji je pokušao snimati pokrete donje vilice i prepoznavao kretanje mandibule napred i bočno. On je bio nazvan „Stomatološki vodič“. Nije zaživeo, a samim tim je i nestao sa scene. Bio je neuspešan jer je bio neprikladan i težak za korišćenje. [8]

„Artikulatori sa fiksnim kondilarnim vođenjem“ i naredni unapređen

Originalni Evensov artikulator, sa funkcijom horizontalne kretnje kondila, može se klasifikovati kao artikulator sa „fiksnim kondilarnim vođenjem“ i on je najraniji od ove vrste. Još dva rana patentirana primera bila su Starr (1868) i Antes-Lewis (1895, 1900). Najpoznatiji artikulator u ovoj kategoriji bio je Bonwill (1858). Svi ovi artikulatori su bili prihvaćeni od strane stomatološke profesije. Zapravo, Bonwill je bio popularan mnogo godina. Ipak, nijedan nije imao principijelni napredak. Tek skoro 50 godina nakon što je Evens bio patentiran, Richmond S. Hayes je sproveo sledeći važan napredak, uvodeći kretanje kondila napred i nadole, kada je izdao svoj patent 1889. god. za „fiksno kondilarno vođstvo“ [8].

Prvi artikulator sa „podesivim kondilarnim vođstvom“

Sledeći važan korak u dizajnu artikulatora dogodio se 1896. godine, kada je William E. Walker iz Pass Christiana, MS, dobio dva patenta za artikulatore sa podesivim kondilarnim vođstvom (Slika 5). Najranija verzija njegovog artikulatora, napravljenog oko 1895. godine, imala je fiksno kondilarno vođstvo. Iako je drugi model pokazivao podesive kondilarne pokrete, oni nisu mogli biti podešeni pojedinačno. Treći model Voker artikulatora bio je prvi koji uključuje pojedinačno podesive kondilarne vodiče, kao i podesive rotacione centre za bočno kretanje. Voker je patentirao ova zadnja dva modela sekvencijalno, nazvavši ih „Vokerovi fiziološki artikulatori“. Da bi izmerio put svakog kondila pojedinačno, Voker je dizajnirao aparat koji je nazvao „klinometer lica“ i postupak za njegovu upotrebu. Budući da je dizajniran da odredi ugao kondilarnih puteva na licu, može se smatrati prvom ekstraoralnom metodom za snimanje pokreta donje vilice, iako putevi nisu zapravo zapisani. Klinometar lica nikada nije bio patentiran [9].

Izgled i rana upotreba incizalnih klinova i vodiča [10]

Već 1840-ih neki oblik „vertikalnog zaustavljanja“ bio je česta komponenta mehaničkih artikulatora. Jasno je da su neki rani pronalazači prepoznali važnost očuvanja vertikalnog odnosa gipsanih modela u artikulatoru i dali tu mogućnost.

Prvi artikulatori koji su patentirani sa povezanim incizalnim klinom i vodičem

C. E. Luce iz Štutgarta u Nemačkoj je 28. novembra 1911. dobio prvi patent za artikulator sa incizalnim pinom i vodičem. Luce je bio jedan od prvih istraživača koji su opisali kretanje kondila nadole i napred (1889). Njegov artikulator je bio prvi koji je imao mogućnost „pisanja“ i kontrolu zadnjeg i incizalnog puta koji su bili funkcionalno generisani (Slika 6).

Drugi artikulator sa ovom karakteristikom je bio patentiran od strane Ernesta Eltnera iz Bazela u Švajcarskoj u julu 1912. godine. Eltnerov artikulator je predstavio horizontalni incizalni vodič sa podesivim zadnjim delom koji ograničava protuzivna kretanja. Inicijalni klin je imao oblik strelice.

Alfred Gysi je dobio patent za svoj „prilagodljivi“ artikulator u oktobru 1912. (Slika 7). Ovo je prvi njegov patent za američki artikulator. Iako je „prilagodljivi“ artikulator bio veoma napredan za to vreme, imao je samo fiksiran 45-stepeni klin za incizalno vođenje. Bez obzira na to, „prilagodljivo“ se smatralo suviše komplikovanim za prosečnog praktičara i on nikada nije postigao komercijalni uspeh. Gysi je koristio precizan dizajn incizalnog vođenja u svom „prostom“ artikulatoru, verziji „prosečne vrednosti“ prilagodljivog artikulatora.

Ray W. Burch of Hart, MI, u decembru 1913. godine primio je četvrti patent izdat za artikulator sa incizalnim klinom. Burch je prvi koji je primenio inovaciju prenošenja maksilarnih i mandibularnih obraznih lukova u artikulator. Obrazni lukovi su bili uređaji povezani sklopom slotova za kondile i podešavani su na licu pacijenta. Inicizalni klin je korišćen prosto za održavanje prednjeg vertikalnog položaja.

Nakon artikulatora Burcha, George B. Snow patentirao je „acme“ artikulator u novembru 1915. godine, koji je bio njegov treći patent, a prvi sa incizalnim klinom i vodičem. U najranijem modelu, protuzioni ugao sekutića podešen je zagrevanjem lema između komponenti identifikovanih brojevima 4 i 8 i pomeranjem stolića gore ili dole. Kasniji modeli su sadržali izmenjive stoliće i, na kraju, podesive stoliće.

David M. Shaw iz Elthama (Engleska) patentirao je artikulator u februaru 1916. Ovaj uređaj je u velikoj meri zanemaren, ali zaslužuje da se pomene zbog značajnog dizajna incizalnog

klina, koji je služio da utiče na protuzivno i bočno kretanje sa zakrivljenim incizalnim klinom tipa strelice koji funkcioniše na krivini vođice.

U novembru 1916. Godine Rupert E. Hall je dobio svoj prvi patent za artikulator, uređaj koji je dobio nadimak „aligator". „Aligator" je bio prvi artikulator koji je uključivao incizalni stolić sa podesivim bočnim krilima. Na drugom Holovom artikulatoru (mart 1917), u revidiranoj verziji „aligatora", incizalni klin je bio dizajniran na stoliću koji je imao ugao od 45 stepeni.

Holov „dentalni okludirajući ram": prvi patentirani stolić incizalnog vođenja sa gotskim lukom

Treći patent artikulatora Ruperta Hola, izdat za „Dentalni okludirajući ram" (april 1917), bio je revolucionaran (Slika 8). To je bio arkon instrument koji se sastojao od podesivih, zakrivljenih kondilarnih puteva, uključujući postavku za Balkwill-Bennettov ugao, kao i mehanizam za podešavanje interkondilarne udaljenosti. Takođe je postojao horizontalni incizalni stolić sa podesivim bočnim krilima, kao i nova karakteristika – trouglasta vođica na svakom bočnom krilu koja se može postaviti tako da prati „gotski luk".

Maksilomandibularni instrument

Dizajnirao ga je Džordž Monson 1918. godine i zasnovan je na teoriji sfere. Prema ovoj teoriji, koja je nastala iz koncepata Monsona i nemačkog anatomiste Grafa fon Špea, mandibularni zubi prelaze preko maksilarnih zuba kao na površinu sfere. Prosečni radijus sfere je četiri inča, ali je Monsonov instrument imao zavrtnje koji su mogli da variraju radijus instrumenta (Slika 9) [5].

Stephen artikulator

Stephen artikulator, razvijen 1921. godine, sličan je u dizajnu Gariotovom artikulatoru osovine iz 1805. godine, osim što ima fiksni nagib kondila i omogućava proizvoljno bočno kretanje. Zadnji zavrtnji drže gornji i donji deo artikulatora na fiksnoj vertikalnoj dimenziji [5].

Artikulator Hanau model C i Kinoskop Hanau model M

Rudolph L. Hanau, inženjer, uticao je na dr Rupert E. Halla da proučava dizajn artikulatora. Početkom 1921. razvio je model pod nazivom artikulator Hanau model C. Godine 1923. razvio je još jedan instrument, artikulator Hanau model M kinoskop (Slika 10). Ovaj zadnji je imao na svakoj strani dvostruke kondile. Unutrašnji delovi su služili u dve svrhe: (1) delovali su kao horizontalni vodiči kondila i (2) kao promenljivi centri rotacije kada se pomeraju unutra ili spolja. Benetov ugao se podešavao rotiranjem ekscentričnog konusa koji se nalazio na spoljnim polovima prema interkonondarnoj osi [5].

Relator Homer

Relator Homer je predstavio 1923. godine Joseph Homer. Bio je zasnovan na principu da se plastični materijal koristi za očuvanje pozicija artikulatora umesto mehaničkih vodiča. Tri čaše u donjem delu artikulatora ispunjene plastičnim materijalom bi snimile zapis i vodile tripodni gornji član u snimljene pozicije. Isti princip je korišćen kasnije u irskim duplukacionim i TMZ instrumentima [5].

Artikulator Wadsworth

Vodsvort je verovao u Monsonovu teoriju sfere, ali nije mogao prihvatiti bilateralnu simetriju kondila. U Vodsvortovom artikulatoru, razvijenom 1924. godine, gipsani modeli su montirani koristeći obrazni luk, a Vodsvortov T-dodatak je određivao treću referentnu tačku. Pregrada je korišćena za merenje udaljenosti od središnje tačke sekutića do centra kondila sa jedne strane. Ova dužina luka je prvo opisana od kondila, a zatim sa središnje tačke sekutića prema standardu koji se nalazio na gornjem delu instrumenta. Presek ovih lukova nalazio se na rotacionom centru za merenu stranu. Centar se koristio za dizajniranje sferične ravni okluzije. Imao je i podesivo interkondilarno rastojanje. Ovo merenje je određeno korišćenjem udaljenosti između kondilskih pokazivača na facijalnom luku minus 0,75 inča rastojanja kože na svakoj strani. Kondilarne staze instrumenta su bile blago zakrivljene [5].

Hanau model H110

Hanau model H110 je predstavio Hanau 1926. godine i dizajniran je prvenstveno za izradu totalne proteze. Obuhvatao je mehaničke proseke mnogih prethodnih koncepata. Imao je pojedinačno prilagodljive kondile u sagitalnoj i horinzontalnoj ravni [5]. Umesto da se koriste bočne snimljene pozicije, bočno podešavanje računato je korišćenjem formule, date u osnovi artikulatora:

$$L = H / 8 + 12$$, gde je H = horizontalni kondilarni ugao.

Modifikovani Hanau H110 model

Dizajniran je 1927. godine i uveo je stočić za incizalno vođenje. Originalni stočić incizalne vođice sa fiksnom krivinom mogao se pomerati samo kao jedinica i nije imao kalibracije za resetovanje. Poboljšani stočić se pojavio na artikulatorima Hanau od 1927. do 1972. godine i omogućio je prilagođavanje u tri dimenzije [5].

Balanser Hagman

Razvijen 1920-ih godina od strane H. C. Hagmana, balanser Hagman otvara i zatvara osovinu koja je u centru uspravne podrške, ali ne zahteva nikakav obrazni luk ili interokluzalni rekord za montažu. Središnji uređaj prenosi maksilarne i mandibularne otiske od pacijenta do artikulatora u jednom koraku. Takođe je zasnovan na sferičnoj teoriji okluzije. Mandibularni zubi su konstruisani prema Špeovoj krivi pomoću balansiranog okluzalnog vodiča, a maksilarni zubi su konstruisani tako da se uklapaju sa mandibularnim zubima [5].

Artikulator Phillips Student

Artikulator Phillips Student (Model C), ili pantografski artikulator, razvio je George P. Phillips 1926. godine (Slika 11). Ovaj artikulator je klasifikovan kao potpuno prilagodljiv, jer je njegov proizvođač tvrdio da će pratiti bilo koji grafički zapis. Phillips

grafički zapisnik je dizajniran tako da u jednom koraku prati gotski luk (iglica) i nagib glenoidne fose. Artikulator je mogao mehanički reprodukovati kretanje grafičkog zapisnika pomoću dva vertikalna klina koji prate horizontalni nagib glenoidne fose i dva horizontalna klina koji su kopirali zapisnik klina [5].

Tripodni instrument Stanbery

Razvijen 1929. godine od strane C. J. Stanberya, ovaj artikulator je dizajniran bez osovine kako bi se olakšala reprodukcija bilo kojeg pozicijskog odnosa. Ne postoji mehanički ekvivalent ili kopija kondila. Artikulator reprodukuje pozicije a ne pokrete. Interokluzalni zapisi centralnog položaja kao i protruzionog, desnog i levog bočnog položaja koriste se za postavljanje tri pojedinačne kupole i otvore stativa, pri čemu se slotovi formiraju ravno do centralne pozicije [5].

Artikulator House

Artikulator House je razvio M. M. House početkom tridesetih godina. Ovaj artikulator koristi intraoralne položaje žvakanja ili druge položaje za podešavanje. Interkondilarni centri rotacije variraju bez pomeranja bočnih stubova koji podržavaju kondilarne elemente, pomoću kukica koje bi mogle kliziti duž interkondilarne trake. Bočno usmeravanje kondila kontrolisano je Benetovim vodičem, pričvršćenim bočno prema urezu za kondile. Stočić incizalnog vođenja mogao je kontrolisati horizontalne i vertikalne pokrete. Bočne ploče u stočiću incizalnog vodiča služile su kao zakrivljeni incizalni klin [5].

Precizni koordinator

Precizni koordinator je razvio W. H. Terrell početkom 1930-ih godina. To je artikulator arkon tipa koji ima krivolinijske vodiče kondila. Dvostruki parabolični brežuljak kontroliše vertikalno i horizontalno prednje vođenje. Incizalni klin je zakrivljen tako da omogućava promene u vertikalnoj dimenziji. Takođe postoji i sloboda kretanja u centralnom odnosu [5].

Artikulator Hanau za krunice i mostove

Njega je proizodio Hanau Inženjering Co. od 1934. do 1971. To je mali artikulator. Zadnji klin i brežuljak je mehanizam za navođenje koji se mogao postaviti tako da simulira radne i balansne ekskurzije od 15 stepeni. Mehanizam se mogao postaviti na L za nadoknade u levim kvadrantima, R za desne kvadrante ili Anterior za prednje nadoknade ili za izjednačavanje desnog i levog pomeranja. Njegove protuzivne kretnje bile su do 30 stepeni. Nije bio potreban obrazni luk [5].

Filipsov okluzoskop

Filipsov okluzoskop razvio je George P. Phillips 1938. godine. Gipsani model gornje vilice je montiran korišćenjem obraznog luka. Artikulator je bio prilagodljiv koriseći intraoralne ili ekstraoralne zapise. Donji član imao je dve podesive jedinice koje su predstavljale dva temporomandibularna zgloba. U okviru svake podesive jedinice postojao je kružni disk koji se mogao naginjati napred-nazad i bočno. Nije imao podesiv incizalni vodič. Inicizalni klin se nalazio na ravnom stočiću jer je Philips verovao da incizalni klin služi samo za sprečavanje zatvaranja i da ne bi trebalo da služi kao treći tempomandibularni zglob [5].

ZAKLJUČAK

Artikulatori su instrumenti koji pokušavaju da reprodukuju opseg kretanja vilica. Prvi dizajni instrumenta imali su za cilj kopirati anatomske odnose ili reprodukovati funkcionalne kretnje vilica. Sofisticiraniji artikulatori su razvijeni zahvaljujući novim saznanjima o anatomiji, mandibularnim pokretima i mehaničkim principima. Međutim, cilj je uvek bio isti: simulirati ili reprodukovati okluzalne odnose ekstraoralno. Bez obzira na to koliko je jednostavan ili komplikovan artikulator, ako ga operator ne koristi pravilno ili nema karakteristike za svrhu za koju se koristi, rezultati će biti razočaravajući [3]. Istorija artikulatora od 1940. godine nadalje biće objavljena u sledećem broju.

Da li ste pažljivo čitali radove?

1. Tehnika 3D štampanje je:
   a) moguće kreiranje skofolda sa složenom strukturom
   b) nemoguće kreiranje skofolda sa složenom strukturom
   c) nemoguće kreiranje bilo kakvih skofolda

2. Oralno-higijenske novine su proveravane kod:
   a) dece obolele od astme
   b) dece obolele od dijabetesa
   c) dece sa posebnim potrebama

3. Najčešće impaktirani zubi su:
   a) donji umnjaci
   b) gornji sekutići
   c) gornji premolari

4. Gasna pena je:
   a) aktuelna tehnika za proizvodnju skofolda
   b) tradicionalna tehnika za proizvodnju skofolda
   c) tehnika koja se razvija

5. Teskoba u donjoj vilici sa impaktiranim premolarom je rešavana kod pacijentkinje uzrasta:
   a) 15 godina
   b) 18 godina
   c) 22 godine

6. Oralno-higijenske navike su analizirane kod:
   a) 72 dece
   b) 96 dece
   c) 136 dece

7. Bioštampači omogućavaju štampanje biomimetičkih skofolda?
   a) Da
   b) Ne
   c) Nemoguće je štampati ćelije

8. Ortodontski tretman kod pacijentkinje sa impaktiranim premolarom je uključivao:
   a) fiksni ortodontski aparat
   b) mobilni ortodontski aparat
   c) i fiksni i mobilni aparat

9. Grupa dece obolela od astme su uzrasta:
   a) 3–9 godina
   b) 6–16 godina
   c) 8–16 godina

10. Hiruško-ortodontska terapija je realizovana kod:
    a) gornjih premolara
    b) donjih premolara
    c) donjih molara

11. Štampanje ćelija i biomaterjala direktno na pacijentu je:
    a) moguće
    b) teško izvodljivo
    c) kompleksno i pod znakom pitanja

12. Oralno-higijenske navike su:
    a) lošije kod dece kontrolne grupe
    b) lošije kod dece obolele od astme
    c) slične kod dece obolele od astme i u kontrolnoj grupi

13. Skofoldi treba da obezbede:
    a) makroskopski strukturni dizajn
    b) mikroskopski strukturni dizajn
    c) makroskopski i mikroskopski strukturni dizajn

14. Deca obolela od astme su prala zube više puta u toku dana:
    a) u 56% slučajeva
    b) u 60% slučajeva
    c) u 77% slučajeva

15. Impaktirani donji desni drugi premolar je doveo:
    a) do resorpcije korena prvog premolara
    b) do resorpcije korena prvog molara
    c) do resorpcije korena i premolara i molara

16. Tehnika laserskog sinterovanja se koristi za skofolde:
    a) vilične kosti
    b) zuba
    c) dugih kostiju

17. Na pojavu impakcije donjih premolara utiču i genetski faktori?
    a) Da
    b) Ne
    c) Skoro nikad

18. Strah od stomatološke intervencije bila je izraženija:
    a) kod dece obolele od astme
    b) kod dece kontrolne grupe
    c) nije bilo razlike između obolele dece i dece kontrolne grupe

19. Impaktirani drugi donji premolar je:
    a) ekstrahovan
    b) ekstrudiran
    c) intrudiran

20. Da bi postupak ekstruzije impaktiranog premolara bio uspešan:
    a) nagib ne sme biti veći od 150
    b) nagib ne sme biti veći od 300
    c) nagib ne sme biti veći od 450

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