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REVIEW

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Slina kao dijagnostička tekućina

Saliva as a Diagnostic Fluid

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Sažetak
Lakoća i jednostavnost te neinvazivan način kojim se slina skuplja od većine ispitanika, mnoge je istraživače upozorila na to da bi mogla, za dijagnostičko ispitivanje, biti prikladnija od krvi. Mogućnosti su predstavljene u znanstvenom radu prezentiranom na Simpoziju oralne biologije, a nakon sastanka Međunarodne udruge za dentalna istraživanja u Nizozemskoj godine 1986. Daljnje analize u proteklom stoljeću nisu dale ništa novoga s obzirom na zaključke iz spomenutoga rada. Sada su, pak, nove, analitičke metode ponovno potaknule zanimanje znanstvenika za dijagnostičku primjenu uzoraka sline. Neke su metode bile predstavljene na sastanku IADR-a godine 2004., a objavljene su sljedeće godine u Advances in Dental Researchu. Točnije, Akademija znanosti grada New Yorka tiskala je tekstove sa simpozija u svojim Analima godine 2007. U toj su publikaciji opisani novi i zanimljivi postupci u dijagnostičkim metodama u kojima se rabi vrlo mala količina sline.

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Ključne riječi
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Uvod

Lakoća i jednostavnost te neinvazivan način kojim se slina skuplja od većine ispitanika, mnoge je istraživače upozorila na to da bi mogla, za dijagnostičko ispitivanje, biti prikladnija od krvi. Mogućnosti su predstavljene u znanstvenom radu prezentiranom na Simpoziju oralne biologije, a nakon sastanka Međunarodne udruge za dentalna istraživanja u Nizozemskoj godine 1986. (1). Daljnje analize (primjerice, referencija 2.) u proteklom stoljeću nisu dale ništa novoga s obzirom na zaključke iz spomenutoga rada. Sada su, pak, nove, analitičke metode ponovno potaknule zanimanje znanstvenika za dijagnostičku primjenu uzoraka sline. Neke su metode bile predstavljene na sastanku IADR-a godine 2004., a objavljene su sljedeće godine u Advances in Dental Researchu (3). Točnije, Akademija znanosti grada New Yorka tiskala je tekstove sa simpozija u svojim Analima godine 2007. U toj su publikaciji opisani novi i zanimljivi postupci u dijagnostičkim metodama u kojima se rabi vrlo mala količina sline (4).

Introduction

The ease by which saliva may be collected from most subjects and the non-invasive nature of the collection have suggested to many research workers that it should provide a more suitable fluid than blood for diagnostic tests. The possibilities were reviewed in a paper given during the Oral Biology Symposium after the meeting of the International Association for Dental Research in Holland in 1986 (1). Subsequent reviews (for example, reference 2) during the last century added little to the conclusions of that paper. In this twenty first century, however, new methods of analysis have led to new possibilities and renewed interest in the diagnostic applications of saliva samples. Some of these were reviewed in a symposium at the IADR meeting in 2004, published in Advances in Dental Research the following year (3). Even more recently, the New York Academy of Sciences hosted a symposium, published in their Annals in 2007. This publication describes new and exciting advances in diagnostic methods using very small volumes of saliva (4).

Slina kao dijagnostička tekućina u doba makroanalize

Opširna istraživanja korelacija salivarnih ionskih koncentracija ili koncentracija specifičnih proteina s oralnim bolestima, nisu uspjela dokazati bilo kakvu korelaciju koja bi mogla imati praktičnu primjenu. Čak i proučavanje sastava sline s antibakterijskom ili antifungalnom djelatnošću nije pokazalo korelacije s bolešću. Becks i Wainwright dokazali su u svojem ranom radu koncentraciju kalcija i fosfata u slini (5), određene korelacije između tih iona i pojavnosti te osjetljivosti pojedinog organizma za zubni karijes, no preklapanje dosega značilo je da postoji vrlo mala mogućnost za predviđanje karijesa. Iako se pokazalo da koncentracije IgA, laktoferina i peroksidaze variraju s pojavnošću zubnog karijesa (6), dosad ipak nisu postale važni parametri u procjeni prognoze bolesti. Koncentracije sekretornih IgA-e u slini vezane su za pojavu karijesa, no prijeklop između ispitanika bez karijesa i onih kod kojih je aktivan, čini taj test bezvrijednim (7). Dosljedno pojavljivanje jednoga salivarnog proteina kod pacijenata oboljelih od Sjoegrenove bolesti (8, 9) pokazalo se nedovoljno karakterističnim i nije zamijenilo analize serumskih antitijela u potvrdi dijagnoze. Doduše, u recentnijim su radovima istraživači došli do naizgled zanimljivih spoznaja: prepoznali su nakupine salivarnih proteinskih markera za oralni rak (10). Moguće korištenje sline kao dijagnostičke tekućine za otkrivanje sustavnih bolesti donedavno je imalo dva velika ograničenja. Prvo, slina se najlakše skuplja ekspektoracijom, a tada je kontaminirana oralnim bakterijama koje dodaju ili uništavaju određene sastavne dijelove sline. Drugo, slina nije jednostavan ultrafiltrat plazme - proizvodi se u sloju epitelnih stanica u acinusima žlijezda, posebnim mehanizmima prijenosa te se inicijalna sekrecija dalje modificira u duktusima. Pasivni tranfer otopljenih tvari iz krvi ili izvanstanične tekućine ograničen je svojstvima slojeva epitelnih stanica acinusa i duktusa (11).

Prije mnogo godina otkriveno je kako je koncentracija glukoze u slini odraz njezine koncentracije u krvi, ali na dosta nižoj razini. Glukoza teško prelazi epitelni sloj i metaboliziraju je stanice epitela, tako da su iznosi konačnih koncentracija u slini oko jedan posto onih u plazmi (12). Iako je bilo pokušaja da se salivarna glukoza uporabi i za dijagnosticiranje i kontroliranje dijabetesa melitusa, nije dovoljno pouzdana da bi potpuno zamijenila mjerenje koncentracija glukoze u plazmi.

Saliva as a diagnostic fluid in an age of macroanalysis

Extensive research on correlations of salivary ion concentrations or specific protein concentrations with oral disease have failed to demonstrate any correlations with practical usefulness. Even studies of components of saliva with antibacterial or antifungal activity have not shown any correlations with disease. The early work of Becks and Wainwright on calcium and phosphate concentrations in saliva (5) showed some correlation of these ions with the incidence of, and the susceptibility to, dental caries but the overlap of the ranges meant that measurements had little predictive value. Similarly, although concentrations of IgA, lactoferrin and peroxidase have each been shown to vary with the incidence of dental caries (6), they have so far proved of little value in assessing the severity or the prognosis of the disease. Concentrations of secretory IgA in saliva are related to caries incidence but the overlap between caries free and caries active subjects renders the test of little use in prognosis (7). A salivary protein found consistently in patients with Sjogren's disease (8,9) proved insufficiently characteristic to compete with the previous serum antibody analyses in confirming a diagnosis. More recent work, however, which is discussed further below, has identified a cluster of salivary protein markers for oral cancer which seem promising (10).

The possible use of saliva as a diagnostic fluid for systemic diseases was until recently subject to two major constraints. Firstly, the saliva most easily obtained is that from expectoration and this is heavily contaminated with oral bacteria which themselves can add to or destroy some components of saliva. Secondly, saliva is not a simple ultrafiltrate of plasma: it is produced across an epithelial cell layer in the acini of the glands by specific transport mechanisms and the initial secretion is further modified in the ducts. Passive transfer of solutes from blood or extracellular fluid is limited by the properties of the epithelial cell layers of acini and ducts (11).

It was found many years ago that salivary glucose concentrations mirrored those in blood, but at a very much lower level. Glucose crosses the epithelial layer with difficulty and is itself metabolised by epithelial cells so that the final concentrations in saliva may be as little as 1% of those in plasma (12). Although attempts have been made to use salivary glucose as a means of diagnosing and controlling diabetes mellitus, these have not proved sufficiently

Učinkovit prijenos određenih iona kroz duktuse žlijezda slinovnica rezultira salivarnim koncentracijama iona sličnim onima u krvi (13).

Izloženost duhanskome dimu praćena je koncentracija salivarnog tiocijanata (14).

Epitelne stanice zapreka su u kretanju tvarima topivima u masti. Dakle, salivarne koncentracije takvih tvari blisko su vezane za njihove koncentracije u krvi. Primjer su steroidni hormoni, ali se kod njih mogu uočiti i neke poteškoće. Hormon poput kortizola ili testosterona putuje krvotokom u dvama oblicima - može biti vezan specifičnim proteinskim nosačem ili nevezan. Jedino njegov nevezani oblik može prijeći u slinu. Budući da nevezani hormon može poticati interakcije s receptorskim stanicama, koncentracija sline odražava količinu aktivnog hormona u plazmi. Ispitivanjem hormona iz plazme dobiva se veća koncentracija, jer je uključen i inaktivan oblik koji se transportira. Drugi steroidni hormoni, poput estrogena, mogu opstati u sulfatiranim i nesulfatiranim oblicima, a samo se nepolarni nesulfatirani oblik pojavljuje u slini. Osim tih ograničenja, slina se pokazala korisnim sredstvom u procjeni nekih poremećaja steroidnih hormona (15, 16).

Opisana je i slična uporaba sline u praćenju koncentracije lijekova u plazmi. Kao i kod steroidnih hormona, liposolubilni lijekovi mogu prelaziti iz žljezdanoga epitela u izlučenu slinu. Dakle, mjerenje karbamazepina u slini može biti rutinska metoda u mjerenju koncentracije lijekova u plazmi (17).

Slina kao dijagnostička tekućina u doba mikro- ili nano-analize

Posljednjih nekoliko godina razvoj kemijskih i fizikalnih metoda omogućio je detekciju neznatnih količina otopljenih tvari u slini i potaknuo nove mogućnosti uporabe u dijagnostici.

Dvodimenzionalna elektroforeza sline, praćena masenom spektrometrijom odvojenih sastavnica, omogućila je kemijsku karakterizaciju bjelančevina i peptida u sekretu. Drugi ključni čimbenik u tom postupku jest razvoj golemih baza podataka u kojima su katalogizirana fiziko-kemijska svojstva bjelančevina i peptida, tako da se mogu prepoznati molekule odvojene u analizi. Ti su novi pristupi rezultirali identifikacijom više od tisuću bjelan-

reliable to displace the measurement of plasma glucose concentration.

Efficient transport of some ions across the ducts of the salivary glands results in salivary concentrations of these ions being parallel to those in blood (13).

Salivary thiocyanate concentrations have been used to monitor exposure to tobacco smoke (14).

Epithelial cells do not provide a barrier to the movement of lipid soluble substances. The concentrations of such substances in saliva are therefore closely related to those in blood. Steroid hormones provide good examples of this but also illustrate some of the difficulties. A hormone such as cortisol or testosterone travels in the bloodstream in two forms – it may be bound by a specific carrier protein, or it may travel in free solution. It is only the free hormone which can pass into saliva. Since the free hormone is that which can interact with receptor cells, the saliva concentration reflects the amount of active hormone in plasma. Assay of the hormone in plasma will give a higher concentration because it will include the inactive form which is being transported. Other steroid hormones, such as the oestrogens, can exist in sulphated or non-sulphated forms and it is only the non-polar non-sulphated form which will appear in saliva. Despite these limitations, saliva has proved a useful medium in the evaluation of some steroid hormone disorders (15, 16).

A similar use of saliva in monitoring plasma drug concentrations has been described. As with the steroid hormones, drugs which are lipid-soluble are able to cross the gland epithelium to reach the secreted saliva. Thus measurement of carbamazepine in saliva can be used as a routine measure of plasma drug concentrations (17).

Saliva as a diagnostic fluid in an age of micro- or nano-analysis

In recent years the development of chemical and physical methods for the detection of minute quantities of solute in saliva have opened up new possibilities for diagnostic uses.

Two-dimensional electrophoresis of saliva followed by mass spectrometry of the separated components has enabled the chemical characterisation of proteins and peptides present in the secretion. The other key factor in this process has been the development of huge databases cataloguing the physico-chemical properties of proteins and peptides so that molecules separated in the analysis can be matched and identified. These new approaches have resulted

čevina i peptida iz sline (18, 19, 20). Peptidni uzorci uključuju i one koje izlučuju same žlijezde, ali su uglavnom derivirani iz staničnih membrana i struktura sekretornih i duktalnih stanica. Trenutačno je stručnjacima izazov u spoznaji mogu li se peptidi ili njihove kombinacije pridružiti oralnim ili sustavnim bolestima. Dosad su objavljene dvije takve primjene. Streckfus (21, 22) je otkrio cERB-2, peptid koji proizvode tumorske stanice egzokrinih žlijezda, a bio je u slini pacijentice s rakom dojke. To, dakle, omogućuje dijagnostički "alat" za pacijentice koje imaju suspektan karcinom dojke. Iako nije ispitano, teoretski bi se moglo očekivati da tumori gušterače, ili čak žlijezda slinovnica, imaju sličnu sekreciju cERB-2. Znači, upitna je specifičnost toga testa u izolaciji. Nekoliko je skupina znanstvenika (10, 23, 24) objavilo da su određene kombinacije salivarnih peptida pridružene karcinomima epitelnih stanica u ustima. Osobito je zanimljiv izvještaj Wonga i suradnika sa Sveučilišta UCLA o interleukinu IL6 (24).

Još je jedna tehnika vrlo važna za razvoj mogućih dijagnostičkih testova – to je imunološko određivanje (immnoassay) - bilo da se radi o fluorescentnim antitijelima ili radiološki označenim sondama. Takve testove možemo umanjiti do određene veličine tako da mogu detektirati i neznatne količine tvari te se rabiti u minijaturnim uređajima koji će biti kasnije opisani.

Razvoj analiza DNK-a i RNK-a te metoda za amplifikaciju njihove količine (poput PCR-a i lančane reakcije polimeraze) omogućio je novu uporabu salivarne analize u dijagnosticiranju i prognoziranju bolesti. Kada su obavljene s minijaturiziranim metodama ispitivanja, mogu se identificirati bakterije i virusi i u neznatnim uzorcima sline (25, 26).

Bez obzira na to koliko je sigurna određena dijagnostička metoda, njezina široka uporaba nije vjerojatna, osim ako su uključene i tehnike koje se mogu primijeniti u rutinskom bolničkom laboratoriju ili kod bolesnikove postelje, ili čak u stomatološkoj ordinaciji. Razvoj tehnologije genskoga čipa (microarray analiza) omogućio je analizu bakterijskih vrsta u slini (26). Tako se sada može obaviti imunološko određivanje na kvadratima akrilamida neznatne veličine koji se impregniraju reagensom nanesenim u točkama, a obrađuju ih posebni čitači. U analizi sline stručnjaci su se koristili tehnikama sličnima onima s DNK, uz mogućnost kemijske amplifikacije signala i eliminacije lažno pozitivnih rezultata. Senzori optičkih vlakana omogućuju učitavanje signala u vrlo preciznim, malim područjima.

in the identification of around one thousand proteins and peptides in saliva (18, 19, 20). The peptide materials identified include those secreted by the glands themselves but the great majority are derived from the cell membranes and structures of the secreting and ductal cells. The challenge now is to see whether any peptides or combinations of peptides can be associated with oral or systemic disease. Thus far two applications have been reported. Streckfus (21, 22) found cERB-2, a peptide produced by cancer cells in exocrine glands, was present in the saliva of patients with mammary carcinoma. This, therefore, provides a diagnostic tool for patients in whom mammary carcinoma is suspected. Although the question has not been investigated, theoretically one would expect pancreatic tumours, or even salivary gland tumours, to give a similar secretion of cERB-2. The specificity of the test in isolation may therefore be questioned. Several groups (10, 23, 24) have reported that particular combinations of peptides in saliva are associated with epithelial cell carcinomas in the mouth. Of particular interest was the report from Wong's group at UCLA in relation to the presence of the interleukin, IL6 (24).

Another technique which has been of great importance in developing possible diagnostic tests has been that of immunoassay, either by radiolabelled or by fluorescent antibody probes. These tests can be scaled down to detect minute amounts of substances and used in the types of miniature devices described later.

The development of DNA and RNA analyses and methods for amplifying their amounts (such as the polymerase chain reaction, PCR) have provided a further way of using salivary analysis in diagnosis and prognosis of disease., .Linked with miniaturised testing methods, these provide ways of identifying bacteria and viruses in minute samples of saliva (25, 26)

However good a diagnostic method may be, it is unlikely to receive wide use unless the techniques involved are capable of translation to the routine hospital laboratory or even the bedside or chairside environment. The development of microarray technology in which immunoassays can be carried out on minute squares of acrylamide get impregnated with spots of reagent and then read with special readers has allowed analysis of bacterial species in saliva (26). Similar techniques with DNA and the possibility of chemically amplifying the signal and eliminating false positives have been used in saliva analysis. Fibre-optic sensors enable the signals to be read in precise small areas.

Zahvaljujući znanju o očekivanim sastavnicama koje prelaze u slinu i onima koje bi mogle pokazati varijaciju u okolnostima specifične bolesti, mogu se oblikovati dijagnostičke metode za kliničku primjenu. Dakle, spoznaja da specifične peptide možemo naći u većim koncentracijama u slini pacijenata oboljelih od bubrežnih bolesti, omogućila je razvoj jednostavnijeg testa na papirnatoj vrpci koja služi za praćenje pacijenata na dijalizi (28).

Slijedile su složenije metode pomoću minijaturiziranih analitičkih sustava koji se drže u ruci, a u njima je slina provedena sustavom duktusa putem kapilarnih sila ili minijaturnih crpki, tako da se mogu obaviti daljnje analize, a rezultiraju fluorescentno obojenim reakcijama. Reakcije očitavaju posebni čitači. Uzorci se u sklopu tih mikrosustava mogu čak i zagrijati ili filtrirati (28-33). Već su dostupni u tvorničkim kompletima kojima se određuju koncentracije bakterije Streptococcus mutans (GC Europe N.V. Belgija), Helicobacter pylori (HMCAP Enteric Products Inc., Stonybrook, NY, SAD), humani imunosupresivni virus (HIV; Orgenics, Inverness Medical Innovations, Yavne 70650, Izrael) i mnogobrojni steroidni hormoni (SalivaTesting, Harrison Street, Sumas, WA98295, SAD).

Zaključak

Jasno je da je istraživanje sline dosegnulo točku u kojoj su moguće dijagnostičke koristi.

With a knowledge of what components may be expected to pass into saliva and those which might show variation in specific disease conditions it becomes possible to design diagnostic methods of use in the clinical situation. Thus the recognition that specific peptides would be found in higher concentration in the saliva of patients suffering from kidney disease enabled the development of a simple paper strip test to monitor patients receiving dialysis on artificial kidney machines (28).

More complex analyses have become possible using handheld miniaturised analysis systems in which a drop of saliva is ducted along capillary tubes either by capillarity or by miniature "pumps" so that stages of analysis can be carried out and result in fluorescent or colour reactions. These can then be read with special readers. Samples can even be heated or filtered within these microsystems (28-32). Already there are available commercial kits allowing estimation of streptococcus mutans concentrations (GC Europe N.V. Belgium), helicobacter (HMCAP Enteric Products Inc., Stonybrook, NY, USA), human immunosuppressive virus (HIV; Orgenics, Inverness Medical Innovations, Yavne 70650, Israel), and a number of steroid hormones (SalivaTesting, Harrison Street, Sumas, WA98295, USA)

Conclusion

It is clear that salivary research has now finally reached a point where diagnostic uses can be realised.

Abstract
The ease by which saliva may be collected from most subjects and the non-invasive nature of the collection have suggested to many research workers that it should provide a more suitable fluid than blood for diagnostic tests. The possibilities were reviewed in a paper given during the Oral Biology Symposium after the meeting of the International Association for Dental Research in Holland in 1986. Subsequent reviews during the last century added little to the conclusions of that paper. In this twenty first century, however, new methods of analysis have led to new possibilities and renewed interest in the diagnostic applications of saliva samples. Some of these were reviewed in a symposium at the IADR meeting in 2004, published in Advances in Dental Research the following year. Even more recently, the New York Academy of Sciences hosted a symposium, published in their Annals in 2007. This publication describes new and exciting advances in diagnostic methods using very small volumes of saliva.

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References

1. Ferguson DB. Current diagnostic uses of saliva. J Dent Res. 1987;66(2):420-4.
2. Malamud D, Tabak LA, editors. Saliva as a Diagnostic Fluid. Conference proceedings. Panama City Beach, Florida, October 22-25, 1992. Ann N Y Acad Sci. 1993;694:1-348.
3. Carter L, editor. Saliva-/Oral-fluid-based Diagnostic Markers of Disease. Proceedings of a Symposium sponsored by the IADR Diagnostic Systems Group, co-sponsored by the IADR Salivary Research and Oral Medicine & Pathology Groups. Honolulu, HI, USA, 12 March 2004. Adv Dent Res. 2005;18(1):1-24.
4. Malamud D, Niedbala RS, editors. Oral-based diagnostics. Festschrift to honor Irwin Mandel and proceedings of a conference. October 10-12, 2006. Lake Lanier Islands, Georgia, USA. Ann N Y Acad Sci. 2007;1098:xiii,1-515.
5. Becks H, Wainwright WW, Young DH. Does salivary calcium and phosphorus composition differ significantly in caries-free and caries-active individuals? J Dent Res. 1941;20(3):171-88.
6. Gråhn E, Tenovuo J, Lehtonen OP, Eerola E, Vilja P. Antimicrobial systems of human whole saliva in relation to dental caries, cariogenic bacteria, and gingival inflammation in young adults. Acta Odontol Scand. 1988;46(2):67-74.
7. Lehner T, Clarry ED, Cardwell JE. Immunoglobulins in saliva and serum in dental caries. Lancet. 1967;1(7503):1294-6.
8. Beeley JA, Khoo KS. Salivary proteins in rheumatoid arthritis and Sjögren's syndrome: one-dimensional and two-dimensional electrophoretic studies. Electrophoresis. 1999;20(7):1652-60.
9. Sreebny LM, Zhu WX. The use of whole saliva in the differential diagnosis of Sjögren's syndrome. Adv Dent Res. 1996;10(1):17-24.
10. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, et al. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res. 2008;14(19):6246-52.
11. Ferguson DB. Oral Bioscience. Gamlingay (United Kingdom): Authors OnLine Ltd.; 2006.
12. Englander HR, Jeffay AI, Fuller JB, Chauncey HH. Glucose concentrations in blood plasma and parotid saliva of individuals with and without diabetes mellitus. J Dent Res. 1963;42:1246.
13. Harden RM, Mason DK, Alexander WD. The relation between salivary iodide excretion and the plasma inorganic iodine concentration. Q J Exp Physiol Cogn Med Sci. 1966;51(2):130-5.
14. Luepker RV, Pechacek TF, Murray DM, Johnson CA, Hund F, Jacobs DR. Saliva thiocyanate: a chemical indicator of cigarette smoking in adolescents. Am J Public Health. 1981;71(12):1320-4.
15. Ferguson DB, editor. Steroid hormones in saliva. Basel: Karger; 1984.
16. Riad-Fahmy D, Read GF, Walker RF, Griffiths K. Steroids in saliva for assessing endocrine function. Endocr Rev. 1982;3(4):367-95.
17. Rylance GW, Moreland TA. Saliva carbamazepine and phenytoin level monitoring. Arch Dis Child. 1981;56(8):637-40.
18. Hu S, Li Y, Wang J, Xie Y, Tjon K, Wolinsky L, et al. Human saliva proteome and transcriptome. J Dent Res. 2006;85(12):1129-33.
19. Denny P, Hagen FK, Hardt M, Liao L, Yan W, Arellanno M, et al. The proteomes of human parotid and submandibular/sublingual gland salivas collected as the ductal secretions. J Proteome Res. 2008;7(5):1994-2006.
20. Xie H, Rhodus NL, Griffin RJ, Carlis JV, Griffin TJ. A catalogue of human saliva proteins identified by free flow electrophoresis-based peptide separation and tandem mass spectrometry. Mol Cell Proteomics. 2005;4(11):1826-30.
21. Streckfus C, Bigler L, Dellinger T, Pfeifer M, Rose A, Thigpen JT. CA 15-3 and c-erbB-2 presence in the saliva of women. Clin Oral Investig. 1999;3(3):138-43.
22. Streckfus C, Bigler L. The use of soluble, salivary c-erbB-2 for the detection and post-operative follow-up of breast cancer in women: the results of a five-year translational research study. Adv Dent Res. 2005;18(1):17-24.
23. Li Y, St John MA, Zhou X, Kim Y, Sinha U, Jordan RC, et al. Salivary transcriptome diagnostics for oral cancer detection. Clin Cancer Res. 2004;10(24):8442-50.
24. Nagler R, Bahar G, Shpitzer T, Feinmesser R. Concomitant analysis of salivary tumor markers--a new diagnostic tool for oral cancer. Clin Cancer Res. 2006;12(13):3979-84.
25. Li Y, Zhou X, St John MA, Wong DT. RNA profiling of cell-free saliva using microarray technology. J Dent Res. 2004;83(3):199-203.
26. Starke EM, Smoot JC, Wu JH, Liu WT, Chandler D, Stahl DA. Saliva-based diagnostics using 16S rRNA microarrays and microfluidics. Ann N Y Acad Sci. 2007;1098:345-61.
27. Smoot LM, Smoot JC, Smidt H, Noble PA, Könneke M, McMurry ZA, et al. DNA microarrays as salivary diagnostic tools for characterizing the oral cavity's microbial community. Adv Dent Res. 2005;18(1):6-11.
28. Walt DR, Blicharz TM, Hayman RB, Rissin DM, Bowden M, Siqueira WL, et al. Microsensor arrays for saliva diagnostics. Ann N Y Acad Sci. 2007;1098:389-400.
29. Li Y, Denny P, Ho CM, Montemagno C, Shi W, Qi F, et al. The Oral Fluid MEMS/NEMS Chip (OFMNC): diagnostic and translational applications. Adv Dent Res. 2005;18(1):3-5.
30. Malamud D, Bau H, Niedbala S, Corstjens P. Point detection of pathogens in oral samples. Adv Dent Res. 2005;18(1):12-6.
31. Gau V, Wong D. Oral fluid nanosensor test (OFNASET) with advanced electrochemical-based molecular analysis platform. Ann N Y Acad Sci. 2007;1098:401-10.
32. Herr AE, Hatch AV, Giannobile WV, Throckmorton DJ, Tran HM, Brennan JS, et al. Integrated microfluidic platform for oral diagnostics. Ann N Y Acad Sci. 2007;1098:362-74.