

THE IMPACT OF DIFFERENT SURFACE CHARACTERISTICS OF FIXED-PROSTHODONTICS RESTORATIVE MATERIALS ON THE EXISTENCE OF PATHOGENIC BACTERIA

ВЛИЈАНИЕ НА РАЗЛИЧНИ КАРАКТЕРИСТИКИ НА МАТЕРИЈАЛИ ЗА ФИКСНО-ПРОТЕТИЧКИ НАДОМЕСТОЦИ ВРЗ ПРИСУСТВОТО НА ПАТОГЕНИ БАКТЕРИИ

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Abstract

Dental plaque on the teeth enamel and surfaces of restorative materials plays an important role in the pathogenesis of oral health. Therefore, there is a great interest in the production of materials that reduce or inhibit dental plaque formation. The purpose of this paper is to present the influence of different surface characteristics of restorative materials on bacterial adhesion through a literature review. Articles published in the electronic bibliographic databases (Medline-Pubmed) have been searched for the following terms: dental plaque or biofilm and restorative materials (composites, porcelain, titanium, Co-Cr alloys, and zirconia), dental plaque or biofilm and surface characteristics, dental plaque or biofilm and surface roughness, dental plaque and surface free energy. Surface characteristics such as surface roughness (SR), surface free energy (SFE), and chemical composition can affect bacterial adhesion and plaque formation. From the literature review it can be concluded that the surface of the materials has a decisive influence on the formation of bacterial plaque and, above all, its roughness. Increasing the coefficient of roughness of the surface and also increasing the surface free energy leads to the formation of biofilm on the surface of the materials. Since papers presented different methodological approaches, the results yielded different and sometimes contradictory outcomes. **Keywords:** dental plaque, biofilm, bacterial cells, restorative materials, surface roughness, surface free energy.

Апстракт

Формирањето дентален плак во емајлот на забите и површините на реставративните материјали игра важна улога во патогенезата на оралното здравје. Затоа, постои голем интерес за производство на материјали кои го намалуваат или го инхибираат формирањето дентален плак. Целта на овој труд е преку литературен преглед да се објасни влијанието на различни карактеристики на различни реставративни материјали врз бактериската адхезија. Во пребарувањето се користени следните елементи: дентален плак или биофилм и реставративни материјали (композици, порцелан, титаниум, легури на Со-Сг, циркониум диоксид), дентален плак или биофилм и површински карактеристики, дентален плак или биофилм и површинска грубост, дентален плак или биофилм и површинска слободна енергија. Карактеристиките на површината на денталните материјали како површинска грубост (SR), површинска слободна енергија (SFE) и нивниот хемиски состав, може да имаат влијание на бактериската адхезија и формирање на плакот. Од литературниот преглед може да се констатира дека површината на материјалите има одлучувачко влијание врз формирањето на бактерискиот плак, а пред сè, неговата грубост. Зголемувањето на коефициентот на грубост на површината, а исто така и зголемувањето на т.н. слободна површинска енергија води до формирање биофилм на површината на материјалите. Бидејќи во трудовите постои голема хетерогеност и различни методолошки пристапи, добиените резултати дадоа различни вредности, а некогаш и контрадикторни. **Клучни зборови:** биофилм, дентален плак, бактериски клетки, реставративни материјали, површинска грубост, слободна површинска енергија.

Introduction

Fixed prosthetic restorations can be made of different materials, such as metal (titanium, chromium - cobalt alloys), types of ceramics, composites, and other contemporary materials.

Particular attention should be given to the type of materials used in the manufacture of fixed-prosthetic restorations, because they are in direct contact with periodontal tissues and can easily compromise their health.

Bacterial accumulation in the gingival margin areas of the tooth and restorative materials is a key factor in

encouraging secondary decay, which is one of the main reasons for the replacement of restorations^{1,2}. Therefore, there is a growing interest in the production of materials that reduce or inhibit dental plaque formation³.

Dental plaque, as an oral biofilm, is recognized as a key factor for decay and periodontal inflammation in humans. Bacterial colonization of dental surfaces or dental materials - such as dental filling materials, dental implants or prosthetic restorations, begins immediately after exposure to the oral environment. In the process of plaque formation, early colonizers, including *Streptococcus sanguinis*, adhere to the salivary layer covering the dental surfaces⁴. This initial adhesion is an important step in the formation of a biofilm that may affect the dental plaque composition.

More than 700 different bacterial species have been found in the oral cavity, of which more than 50% cannot be cultivated. Microflora of the teeth, tongue, buccal epithelium, soft and hard palate, and vestibulum consists 20-30 different dominant species at each site, and the number of dominant species per individual ranges from 30 to 70. The most common species belongs to *Gemella*, *Granulicatella*, *Streptococcus* and *Veillonella*^{5,6}.

A number of factors have been identified that influence the formation of biofilm, such as surface roughness and surface free energy. Microscopic studies of early dental plaque formations have shown adhesion of the initial colonized bacteria along the cracks and pits in the enamel, indicating the influence of surface structure on bacterial adhesion⁷. Mjor et al.⁸ have reported that the margins of dental restorations stimulates bacterial recolonization and acid production as metabolic substances of cariogenic bacteria.

In addition, many studies have found that there is a variation in the effect of different types of restorations on the growth of specific bacteria on dental plaque according to its material composition^{9,10,11}.

Various studies have shown that restoration margins have always been suitable sites for plaque accumulation and reproduction of bacteria that results with gingival inflammation and even tooth loss in some cases. Restoration materials and cement can affect periodontal tissues in different ways and result in gingivitis and gingival damage. These developments usually occur in restorations with subgingival margins and may be due to physical or chemical characteristics of the materials. In most cases, these destructive reactions are due to the roughness of the surfaces of dental materials rather than their composition¹². In such cases the rough surface results in gingivitis and accumulation of more plaque.

The literature emphasizes the fact that besides the biochemical factor, the non-specific physio-chemical factor also plays an important role in the adhesion phe-

nomenon. From the physio-chemical aspect, the phenomenon of bacterial adhesion is explained by two theories: thermodynamic and classical. The thermodynamic theory of bacterial biofilm formation is explained by the action of free surface energy (SFE) between adjacent surfaces and liquids. In contrast, classical DLVO theory (Derjajung, Landau, Verwey, Overbeek) explains the mechanism of biofilm formation as a long-term reaction between a bacterial cell and the tooth surface due to attractive van der Waals forces and repulsive electrostatic forces¹³.

Aim of the paper

The purpose of this paper is to evaluate and compare the influence of surface characteristics of restorative materials such as surface roughness, surface free energy, and surface chemistry on bacterial adhesion of different restorative materials used in making fixed prosthetic restorations.

Materials and methods

Articles published in the electronic bibliographic databases (Medline-Pubmed) have been searched for the following terms: dental plaque or biofilm and restorative materials (composites, porcelain, titanium, Co-Cr alloys, and zirconia), dental plaque or biofilm and surface characteristics, dental plaque or biofilm and surface roughness, dental plaque and surface free energy. In vitro and in vivo studies are included in this study. The papers contained great heterogeneity, different methodological approaches and outcome changes.

Surface Roughness

Surface roughness (SR) measurement is an important aspect in determining the surface properties that influence biofilm formation. Different techniques can be used to evaluate this parameter. Research on this topic includes qualitative assessments [atomic force microscope (AFM) and scanning electron microscopy (SEM)] and quantitative methods (2D and 3D surface analysis profile). Quantitative profile analysis can be performed with a contact diamond laser and a non-contact laser¹⁴.

Because surface topography is three-dimensional in nature, 3D surface topography measurement provides a more realistic analysis of the surface and gives a complete description of the surface topography. Laser or white light profilometry enables the three-dimensional study of the specimen's (or material's) surface without any contact¹⁵.

For the purpose of qualitative evaluation, measurement is usually used to observe the scratches and imperfections of material surfaces. However, SEM is a limitation in defining surface topography, as it only allows morphological evaluation of the sample surface¹⁶.

For visual and high resolution qualitative analysis of surface topography, the use of AFM seems more appropriate. In addition to SEM, AFM can offer more detailed topography of the surface, providing three-dimensional surface analysis in nanometric resolution.

Surface Free Energy

Surface Free Energy (SFE) is described as all solid surface energy equivalent to the liquid surface voltage. It is defined¹⁷ as “the work required to increase the surface area of a substance by 1cm²” and is an important factor in determining surface reactivity. Several different approaches can be used to determine SFE by measuring the angle of contact (θ) formed by different liquids (distilled water, ethylene glycol, and glycerol) that are different in hydrophobicity on a certain surface.

Chemical Composition

The chemical composition of the dental material will further affect the bacterial adhesion since both proteins and microorganisms can chemically attach or get attracted to components in the material, by van der Waal forces, acid-base reactions or electrostatic interactions¹⁸. In most patients, there will be several different materials present in the mouth simultaneously which can interfere with the biofilm formation and the microbiota in general. The chemical interaction between material and microorganisms can lead to alterations in the surface properties over time¹⁹.

In vivo and in vitro experiments to study the development and adhesion of bacteria

In-vivo experiments by Glantz²⁰ in 1969 show that surfaces characterized by high free surface energy (SFE) are more susceptible to bacterial adhesion. Research by Quirynen et al.²¹ show a correlation between the value of free surface energy and the amount of plaque. Low-SFE surfaces are characterized by less mature supra - and subgingival plaque. Comparing the interdependence between the free surface energy and the degree of surface roughness, it has been shown that the degree of surface roughness is an important factor for bacterial adhesion.

Imgard Hauser G et al.⁴ performed an in vitro study that aimed to compare the adhesion of Streptococcus

sanguinis on integrated dental implants and restorative materials versus human tooth enamel, and also to determine the viability of bacteria that initially adhere. In their study, the testing materials were titanium, gold, ceramics, and composites. Rectangular test specimens were used and polished. Surface roughness was measured with a Hommel tester. The viability of the adhered bacteria was estimated using a double fluorescence staining method that allows differentiation of vital and non-vital bacterial cells according to Decker²². The surface roughness of the tested materials was $Ra = 0.24 \mu$ which corresponds to the average roughness of the enamel surface.

The obtained results showed differences in cell adhesion and vitality of the adhered cells, thereby indicating different characteristics of the substrate material. It has been shown that the physical and chemical properties of the materials - such as surface free energy, hydrophobicity and roughness, as well as the composition (composition) of the material, influence the initial bacterial adhesion^{3, 23}. The number of adhered Streptococcus sanguinis cells per mm² was significantly greater in surfaces of titanium, gold, and ceramics than in enamel, whereas bacterial adhesion in the composites was significantly lower, and their vitality was lower compared to those found on the enamel surface. The percentage of adhered vital Streptococcus sanguinis was higher in enamel (92.5%) whereas it was significantly lower in all four tested restorative materials (41.5-69.1%). These results are in accordance with other studies^{24, 25, 13}. However, it was noted that fewer bacteria were retained in the composites, although the hydrophilic properties of the surfaces were similar to the other materials tested. In addition, some dental restorations release metal ions or fluorides in the medium - with a possible impact on the vitality of the adhered bacteria^{22, 26}. This may enhance the explanation for the low percentage of vital adherent cells in the restorative materials used in this study.

Bulem Yzugullu et al.²⁷ in their study investigated the effect of feldspar porcelain surface treatment on the adhesion of Streptococcus mutans. Ninety-six porcelain specimen discs were fabricated and divided into six equal groups according to surface treatment: group 1 - fine-grit diamond polishing, group 2 - self-glazing, group 3 - overglazing, group 4 - overglazing followed by a finishing procedure and then overglazing, group 5 - Pearl Surface polishing and group 6 - Diamond Twist SCLTM polishing. Mean Ra values and standard deviation of porcelain samples after different surface treatments showed statistically significant disparities in surface roughness. The contact angle was also influenced by the procedures used to process the surfaces. The high values of the contact angle led to all the specimens hav-

ing hydrophobic surface properties. The group of specimens glazed twice after grinding (group 4), which largely eliminated surface irregularities and reduced surface roughness values ($R_a = 0.8 \mu\text{m}$), showed the lowest bacterial adhesion, while the highest bacterial adhesion value was present in the group of specimens having the highest surface roughness value ($R_a = 1.6 \mu\text{m}$) (group 1). Reglazing after grinding may therefore decrease bacterial adhesion beneficially.

Quirynen and Bollen²³ suggest that surface roughness and surface free energy are the major factors affecting bacterial adhesion. They further show that the influence of surface roughness is greater than the surface free energy and surface hydrophobicity. Generally, roughness of surfaces promotes bacterial adhesion while smooth surfaces minimize it.^{23,28} According to Bollen et al.²⁹ roughness of surfaces less than $R_a = 0.2 \mu\text{m}$ has no quantitative and qualitative effects on bacterial adhesion. In addition, variations around this value have a negligible effect on bacterial adhesion. In this study, the roughness of all samples is about $0.2 \mu\text{m}$, hence, it turns out that differences in bacterial adhesion cannot be explained in terms of surface roughness. This would mean that any observation of differences in bacterial adhesion is likely due to other properties of the surface and the composition of the used materials.

The literature emphasizes the fact that on the surface of restorative materials, there is greater accumulation of plaque and retention of bacteria than on the surface of enamel^{10, 11}. The amount and composition of bacterial biofilm in prosthetic restorations has been found to vary and depend on the type of restorative materials³⁰. Restoration materials such as ceramics, composites, titanium, and Cr-Co-Mo alloys have been tested in chambers with special conditions that imitate the oral cavity (laminar flow chamber). The adhesion of *Streptococcus mutans* showed significant differences between different materials. The highest values of bacterial adhesion were found in composite samples, while the lowest values of bacterial adhesion were found in chromium-cobalt alloys and titanium restorations. *Streptococcus mutans* showed moderate adhesion to ceramic specimens which was larger than the alloys and lower than the composites. According to some authors, these result values are due to the bacteriostatic properties of the metals used³⁰. This findings are contrary to the findings of Imgard Hauser et al.⁴.

Jalalian et al.³¹ evaluated the adhesion of *Streptococcus mutans* to zirconia, feldspar porcelain, titanium alloys, and indirect composite resin. The study used 10 samples (5mm diameter and 1mm thick) of each material. The enamel was used as a reference value. Bacterial adhesion was determined using a scanning electron

microscope. The results showed the highest bacterial adhesion in the composite specimens, while the lowest bacterial adhesion was observed in the zirconia specimens. The effect of surface roughness was also studied, but no correlation was found between surface roughness and bacterial adhesion. Yet, another study conducted by Yu et al.³² showed that increased surface roughness of zirconia and its hydrophobicity resulted in increased surface forces of adhesion and early attachment of *Streptococcus mutans*.

The density and morphological aspects of the biofilm adhered to different prosthetic restoration materials were investigated by Julio et al.³³, using 60 cylindrical specimens divided into four groups: porcelain, Co-Cr alloy, titanium, and zirconium. Procedures were then performed in a microbiological laboratory for the cultivation of biofilm in human saliva. The unit of measurement used for counting colonies on surfaces was CFU/cm², analyzed by spectrophotometry (absorption) and scanning electron microscopy (FEG-SEM). The highest absorption values and number of CFU/cm² colonies were recorded in biofilms grown in Co-Cr alloys within the first 24 hours and after 48 hours, compared to the other materials used in the study. FEG-SEM images also showed higher biofilm density in Co-Cr alloys.

The results of this study show that ceramic surfaces induce a low density of biofilm associated with a small number of colonies. This may be related to the low level of free energy intensity found on ceramic surfaces. Also, titanium-developed biofilm reveals low density, which can be explained by the presence of passive titanium oxide film (mainly TiO₂). Biofilm morphology was also observed by scanning electron microscopy, which showed a lower biofilm growth after 48 hours in porcelain and zirconia compared to titanium. The biofilm that developed on zirconia and titanium also showed a slight increase in density after 48 h growth compared with that of 24 h growth in relation to the same material. However, porcelain biofilm density remained stable for periods of 24 and 48 hours. In fact, the present results indicate a trend towards higher accumulation of oral biofilms of prosthetic structures based on CoCr alloys when compared to those based on titanium or zirconia.

In most studies, human tooth enamel was treated as a place with the lowest adhesion level of dental plaque compared to restorative materials, but in an in vitro study by Jalalian et al.³⁴ comparing the adhesion level of *Streptococcus mutans* in polished IPS e. max, feldspar porcelain and enamel met different results. The study was conducted in vitro. Porcelain samples were polished with an ultradent $0.5 \mu\text{m}$ diamond polish for 60 seconds while the enamel samples were not polished. They were

then exposed to a standard bacterial suspension of *Streptococcus mutans* in a microbiological laboratory at a concentration of 1×10^6 mg/ml. The results showed higher adhesion of *Streptococcus mutans* to enamel samples, while the lowest value of adhesion were encountered in IPS e.max.

Conclusion

From the literature review it can be concluded that the latest research on bacterial adhesion to materials used for dental reconstruction, the surface of the material has a decisive influence on the formation of bacterial plaque, and above all its roughness. Increasing the surface roughness coefficient above the Ra value of $0.2 \mu\text{m}$ and increasing the surface free surface energy leads to the formation of biofilm on the surface of materials.

However, evaluation of surface chemistry may also be suitable for studying the biological behavior of restorative materials. Restorative materials submitted in different surface treatment protocols may show similar roughness and SFE values, but a different chemical surface composition may affect their biological performance.

Dental ceramics is a material that has the least ability to absorb bacteria on its surface compared to other materials. Comparing different types of ceramics, zirconia is the material with the lowest degree of bacterial adhesion.

Since the cited articles presented different methodological approaches, the results yielded different and sometimes contradictory outcomes.

Reference

1. Deligeorgi V., Mjor IA., Wilson NH. An overview of reasons for the placement and replacement of restorations. *Prim Dent Care*. 2001; 8:5-11.
2. Fontana M., Gonzalez-Cabezas C. Secondary caries and restoration replacement: an unresolved problem. *Compend Contin Educ Dent*. 2000; 21:15-18.
3. Boeckh C., Schumacher E., Podbielski A., Haller B. Antibacterial activity of restorative dental biomaterials in vitro. *Caries Res*. 2002; 36:101-107.
4. Hauser-Gerspach I, Kulik EM., Weiger R., Decker EM., Von Ohle C., Meyer J. Adhesion of streptococcus sanguinis to dental implant and restorative materials –in vitro. *Dent Mater J*. 2007; 26(3): 361-6.
5. Mager DL., Ximenez-Fyvie LA., Haffajee AD., Socransky SS. Distribution of selected bacterial species on intraoral surfaces. *J Clin Periodontol*. 2003; 30:644-654.
6. Aas JA., Paster BJ., Stokes LN., Olsen I., Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005; 43:5721–5732.
7. Lee BC, Jung GY, Kim DJ, Han JS. Initial bacterial adhesion on resin, titanium and zirconia in vitro. *J Adv Prosthodont*. 2011; 3: 81-4.
8. Mjor IA. The frequency of secondary caries at various anatomical locations. *Oper Dent*. 1985; 10:88-92.
9. Mjor IA, The reasons for replacement and the age of failed restorations in general dental practice. *Acta Odontol Scand*, 1997; 55:58-63.
10. Beyth N, Domb AJ, Weiss EI. An in vitro quantitative antibacterial analysis of amalgam and composite resins. *J Dent*. 2007; 35:201-206
11. Satou J, Fukunaga A, Satou N, Shintani H, Okuda K. Streptococcal adherence on various restorative materials. *J Dent Res*.1988; 67:588-591.
12. Willershausen B, Kottgen C, Ernst CP. The influence of restoration materials on marginal gingival. *Eur j Med Res*.2001; 29; 6(10): 433-9.
13. Teughles W, Van Assche N, Sliepen I. Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. *Clin Oral Imp Res*. 2006; 17(supl. 2):68–81.
14. Wassell RW, McCabe JF, Walls AW. Wear characteristics in a two-body wear test. *Dent Mater*. 1994; 10:269-274.
15. Park JW, Song CW, Jung JH, Ahn SJ, Ferracane JL. The effects of surface roughness of composite resin on biofilm formation of *Streptococcus mutans* in the presence of saliva. *Oper Dent* 2012; 37:532-539.
16. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigão J, Lambrechts P, Vanherle G. Microscopy investigations. Techniques, results, limitations. *Am J Dent* 2000; 13:3D-18D.
17. Ahn SJ, Lim BS, Lee SJ. Surface characteristics of orthodontic adhesives and effects on streptococcal adhesion. *Am J Orthod Dentofacial Orthop*. 2010; 137:489-495.
18. Sakaguchi, R.; Powers, J. *Craig's Restorative Dental Materials*, 13th ed.; Elsevier: Philadelphia, PA, USA, 2012.
19. Øilo M, Bakken V. Biofilm and Dental Biomaterials, *Materials* 2015; 8: 2887-2900; doi:10.3390/ma8062887.
20. Glantz. P.O. On wettability and adhesiveness, *Odont Revy*. 1969; 20 (suppl. 17), 5-124.
21. Quirynen M, Marechal M, Busscher HJ, Weerkamp AH, Darius PL, van Steenberghe D. The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. *J Clin Periodontol*. 1990 Mar; 17(3):138-44.
22. Decker EM. The ability of direct fluorescence- based two colour assays to detect different physiological states of oral streptococci. *Lett Appl Microbiol*. 2001; 33:188-192.
23. Quirynen M, Bollen CM. The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *J Clin Periodontol* 1995; 22:1-14.
24. Takatsuka T., Konishi N., Nakabo S., Hashimoto T Torii Y., Yoshiyama M. Adhesion in vitro of oral streptococci to porcelain, composite resin, cement and human enamel. *Dent Mater J* 2000; 19:363-372.
25. Grivet M., Morrier JJ., Benay G., Barsotti O. Effect of hydrophobicity on in vitro streptococcal adhesion to dental alloys. *J Materials Sci: Mater Med* 2000; 11:637-642.
26. Tanner J., Robinson C., Söderling E., Vallittu P. Early plaque formation on fibre-reinforced composites in vivo. *Clin Oral Invest*. 2005; 9:154–160. English.
27. B. Yuzugullu, C. Celik, TB Ozcelik, S Erkut, P Yurdakul, Y Ocal, B Sener. The effect of different polishing sequences on the adhesion of streptococcus mutans to feldspatic porcelain. *The Journal of Adhesion*, 2016; 92(12):939-49.
28. Sardin Morrier j-j, Benay G, Barsoti O. In vitro streptococcal adherence on prosthetic and implant materials. Interactions with psychochemical surface properties. *J Oral Rehab*. 2004; 31:140-148.
29. Bollen CM, Papaioannou W, Van Eldere J, Schepers E, Quirynen M, Van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and periimplant mucositis. *Clin Oral Impl Res*. 1996; 7: 201-211.

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30. Rosentritt M, Hahnel S, Gröger G, Mühlfriedel B, Bürgers R, Handel G. Adhesion of Streptococcus mutans to various dental materials in a laminar flow chamber system. *J Biomed Mater Res B Appl Biomater*. 2008 Jul; 86(1):36-44.
 31. Jalalian E, Mostofi ShN, Shafiee E, Nourizadeh A, Nargesi RA, Ayremlou S. Adhesion of Streptococcus mutans to Zirconia, Titanium Alloy and some other Restorative Materials: "An in-vitro Study". *Advances in Bioscience and Clinical Medicine*. 2015; 3(2):13-20.
 32. Pei Yu, Chuanyong Wang, Jinglin Zhou, Li Jiang, Jing Xue, and Wei Li. Influence of Surface Properties on Adhesion Forces and Attachment of Streptococcus mutans to Zirconia In Vitro. *BioMed Research International*. Volume 2016 (2016), Article ID 8901253, 10 pages.
 33. Júlio C. M. Souza, Raquel R. C. Mota, Mariane B. Sordi, Bernardo B. Passoni, Cesar A. M. Benfatti, Ricardo S. Magini. Biofilm Formation on Different Materials Used in Oral Rehabilitation. *Brazilian Dental Journal* (2016) 27(2): 141-147.
 34. E. Jalalian, GH. Mofrad, M. Rahbar, A. Mohseni, M. Mohebbi. In Vitro Adhesion of Streptococcus Mutans to Polished IPS e.max and Feldspathic Porcelain. *Journal of Islamic Dental Association of IRAN (JIDAI)* Autumn 2015; 27, (4).