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Using the Response Surface Methodology for Periodontitis Diagnosis

Uso da Metodologia de Superfície de Resposta para Diagnóstico de Periodontite

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Abstract

Response surface methodology (RSM) consists of mathematical and statistical techniques to develop models which help to understand the influence of various factors on a dependent variable of interest. The feasibility of RSM use to detect cases of periodontitis and its correlated factors has not yet been evaluated. This study developed mathematical models for periodontitis diagnosis independent of periodontal probing using the RSM. Demographic, socioeconomic, behavioral, systemic, local factors, and periodontitis were assessed in 176 volunteers. Periodontitis case was defined according to three different definitions: 1) ≥3 sites with clinical attachment level (CAL) ≥4 mm; 2) at least one site with CAL ≥4 mm and bleeding on probing; 3) ≥2 proximal sites with CAL ≥3 mm and ≥2 proximal sites with probing depth (PD) ≥4 mm (not on the same tooth) OR 1 site with PD ≥5 mm. 4th-degree polynomial equations showed high coefficients of determination (R^2= 1) and were used to represent the mathematical models of periodontitis cases. According to definition 1, the diagnosis of periodontitis was accurate by including in the model: age, sex, education level, plaque index (PI), number of missing teeth, previous hygiene instructions, and body mass index (BMI). According to definition 2, the diagnosis of periodontitis was accurate by including in the model: age, sex, education level, income, PI, previous oral hygiene instructions, frequency of brushing and type of toothbrush, and use of mouthwash in the model. For an accurate diagnosis of periodontitis according to definition 3, the model included: age, education level, IP, number of missing teeth, previous oral hygiene instruction, BMI, and diabetes. The multifactorial mathematical models were able to diagnosis periodontitis according to different periodontitis case definitions using only variables of easy evaluation and non-invasive.

Keywords: Periodontal Diseases. Diagnosis. Theoretical Model. Logistic Models.

Resumo

A metodologia de superfície de resposta (MSR) consiste em técnicas matemáticas e estatísticas para desenvolver modelos que ajudam a entender a influência de vários fatores em uma variável dependente de interesse. A viabilidade do uso da MSR para detectar casos de periodontite e seus fatores correlacionados ainda não foi avaliada. Este estudo desenvolveu modelos matemáticos para diagnóstico de periodontite independente da sondagem periodontal usando a MSR. Fatores demográficos, socioeconômicos, comportamentais, sistêmicos, locais e periodontite foram avaliados em 176 voluntários. O caso de periodontite foi definido de acordo com três definições diferentes: 1) ≥3 locais com nível de inserção clínica (NIC) ≥4 mm; 2) Um local com NIC ≥4 mm e sangramento à sondagem; 3) ≥2 locais proximais com NIC ≥3 mm e ≥2 locais proximais com profundidade de sondagem (PS) ≥4 mm (não no mesmo dente) OU 1 local com PS ≥5 mm. Equações polinomiais de 4º grau apresentaram altos coeficientes de determinação (R^2= 1) e foram utilizadas para representar os modelos matemáticos dos casos de periodontite. De acordo com a definição 1, o diagnóstico de periodontite foi preciso ao incluir no modelo: idade, sexo, escolaridade, índice de placa (IP), número de dentes perdidos, instruções de higiene anteriores e índice de massa corporal (IMC). De acordo com a definição 2, o diagnóstico de periodontite foi preciso ao incluir no modelo: idade, sexo, escolaridade, renda, IP, instruções prévias de higiene bucal, frequência de escovação e tipo de escova dental e uso de enxaguatório bucal no modelo. Para um diagnóstico preciso de periodontite de acordo com a definição 3, o modelo incluiu: idade, escolaridade, IP, número de dentes perdidos, instrução prévia de higiene oral, IMC e diabetes. Os modelos matemáticos multifatoriais foram capazes de diagnosticar a periodontite de acordo com diferentes definições de casos de periodontite usando apenas variáveis de fácil avaliação e não invasivas.

Palavras-chave: *Doenças Periodontais. Diagnóstico. Modelo Teórico. Modelos Logísticos.*

1 Introduction

Full-mouth periodontal examinations are considered the gold standard to determine the periodontal status of an individual[1]. This evaluation is based on the measurement of distance from the gingival margin to the coronal aspect of the junctional epithelium and the history of attachment loss up to the point in time of the examination [2]. However, a complete mouth examination can be time-consuming and resource-intensive for dental hygienists and dentists and distressful for the patient. Furthermore, many factors can influence that measurement, such as probing reproducibility, probing force, probe angulation, gingival health status, and probe type[2].

Surveys in different areas have used the response surface methodology (RSM) to optimize processes and reduce the cost and time of experiments[3-8]. The RSM consists of mathematical and statistical techniques to develop an adequate functional relationship between a response of interest and several associated (or input) variables[9]. RSM allows computer simulations to be carried out, involving many variables that simultaneously show complex interactions among them. The result of these simulations provides optimized models, capable of determining the influence of several variables on the results of the system or process under analysis [5]. In medical research, especially in anesthesiology and pharmacology, RSM has been used to analyze drug interactions, pharmacodynamics and toxicity[3,4,6-8]. RSM has been applied to find drug combinations that increase efficacy, decrease doses while keeping the same effectiveness, and decrease toxicity[6,8].

To our knowledge, RSM has not been used to identify factors correlated to periodontitis or to create mathematical models for periodontal diagnosis. This study developed multifactorial mathematical models for periodontal diagnosis based on three different case definitions of periodontitis, considering only variables of easy and non-invasive evaluation by using RSM.

2 Material and Methods

This study was conducted following the World Medical Association Declaration of Helsinki and was approved by the Brazilian Research Ethics Committee of the Ministry of Health, Brasilia, Brazil (register no. 505/2011; process no 25000.066822/2011-45).

2.1 Data collection

Two hundred twenty individuals aged 19 years and older from the state of Bahia, in Northeast Brazil, were assessed. Medical exclusion criteria included conditions that require the use of antibiotics before periodontal probing. However, none of the individuals met the exclusion criteria.

The fieldwork was performed between 2011 and 2017. Before the periodontal examination, in-person interviews were conducted by 2 trained nurses to collect standard data on demographic and socioeconomic status as well as other habits and health-related data using a structured written questionnaire. A fasting blood glucose test was performed on all individuals (OneTouch Ultra Mini, Lifescan, Milpitas, CA, USA), and the height and weight were measured (kilograms per meter squared). Bodyweight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using an anthropometric scale (Welmy, São Paulo, Brazil). Patients were required to stand barefoot, wear light clothes, and look out into the Horizon.

Four calibrated periodontists, assisted by four trained undergraduate students from the Dental School of the Federal University of Bahia, performed the clinical examinations. The intraclass and interclass correlation coefficient (ICC) values revealed intraexaminer and interexaminer reproducibility on site level (Intraexaminer: ICC ≥ 0.81; Interexaminer: ICC ≥ 0.75).

Clinical examinations were performed using a headlight (Turboled, Nautika, São Paulo, São Paulo, Brazil) with the individuals seated on regular chairs in schools and health care facility offices.

All permanent fully erupted teeth, except the third molars, were examined using a manual periodontal probe (PCP-UNC 15, Hu-Friedy, Chicago, IL, USA). Probing depth (PD) and gingival margin level relative to the cementoenamel junction (CEJ-GM) were measured at 6 sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual). Measurements were made in millimeters and rounded to the next whole millimeter. Clinical attachment level (CAL) was calculated as the sum of the PD and CEJ-GM measurements. Plaque index (PI) was defined as visible dental plaque/biofilm[10].

2.2 Data analysis

Data analysis was performed using modeFRONTIER software (ESTECO North America, Miami, United States of America) to determine the model/mathematical equation that best explains the observed relationship between periodontitis and input variables analyzed.

Three output variables were established based on definitions of the case of periodontitis: 1) 3 or more sites with CAL ≥ 4 mm[11]; 2) at least 01 site with CAL ≥ 4 mm, and bleeding on probing[12]; 3) ≥ 2 proximal sites with CAL ≥ 3 mm and ≥ 2 proximal sites with PD ≥ 4 mm (not on the same tooth) OR 1 site with PD ≥ 5 mm[13] .

Fifteen input variables were included in the mathematical model as follows: age, sex, education level, income level, plaque index (PI), number of missing teeth, nicotine dependence, alcohol dependence, body mass index, hypertension, diabetes, frequency of brushing and flossing, type of toothbrush, use of mouthwash, and previous oral hygiene instruction. Age, education level, monthly income, number of missing teeth, body mass index were analyzed as continuous variables. Frequency of brushing and flossing were classified as at least once daily or less than once. Type of toothbrush was classified as soft or hard. Daily use of mouthwash was categorized as yes or no. Body mass index (BMI) was calculated as weight divided by height squared (kilograms per meter squared)[14]. Individuals were classified as current non-smokers or smokers. PI was classified as < 40% or ≥ 40% (adapted from Torrungruang et al. 2005)[15]. Individuals were considered diabetic by self-reported physician diagnosis or when fasting blood glucose ≥126 mg/dL was associated with diabetes symptoms (increased thirst, increased urination, and unexplained weight loss). Otherwise, they were considered non-diabetic. Hypertension was classified as: SBP ≥ 139 and DBP ≥ 89 mmHg; or non-hypertension systolic blood pressure (SBP)[16]. According to the Fagerstrom Test for Nicotine Dependence[17] and the Alcohol Use Disorders Identification Test *(*AUDIT)[18], the individuals were categorized as nicotine or alcohol dependent, respectively.

After importing the data from excel to modeFRONTIER software, cases with missing data were excluded. Thus, from an initial sample of 224 subjects, only 176 cases were considered for the statistical analysis. The evaluation of the correlation between input and output variables was performed by RSM. In the first stage of the analysis, the algorithm Smoothing Spline ANOVA" (SS-ANOVA) screened input variables in all output variables. Based on the results of the correlation matrix provided by the SS-ANOVA algorithm, a diagram was generated with the relative weight of the input variables. Thus, indicating the degree of relevance of each variable involved in the process. The independent variables are displayed on the x-axis of the diagram. The weight of the contribution of each factor to the answer is recorded on the y-axis. The sum of all values is equal to 1.

The second stage was of polynomial regression. At this stage, the program defined the coefficients of the equations and created polynomial equations. This is the step responsible for the reduced number of input variables. The third and final stage was to assess the representativeness of the model. At this stage, we evaluated the coefficients of determination (R^2) and the residue curves. These are the parameters responsible for checking whether the model fit is appropriate. The R^2 measures how much the model explains the dependent variable. The higher R^2 value, the more it can explain the variation in the response variable. The evaluation of the residue curves is essential to know if the regression analysis results produced a fitted model.

All these meta-model polynomial analyses were repeated excluding the input variables with less relevance, by considering the SSANOVA screening, one by one, until finding the equation that best represented the model.

3 Results and Discussion

All cycles of meta-model polynomial analysis showed that the 4th degree polynomial equations had the highest coefficients of determination (R^2= 1) and a maximum absolute error equal to or close to zero in all multifactorial models. This result could demonstrate the relationship between the output (Periodontitis Case) and input variables. Agreement between the clinical periodontal diagnosis and the result of the mathematical model was observed in 100% of the 176 individuals.

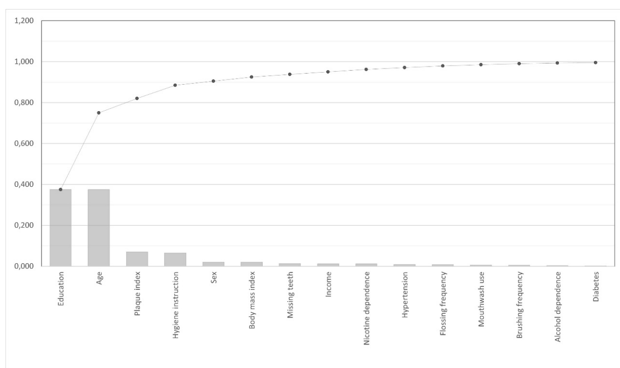
Tables 1, 2 and 3 show the effect of the factors in the measured output variable. The figures 1, 2, and 3 illustrate the participation of each input variable established by SSAnova screening in each outcome analyzed. According to case definition 1, periodontitis diagnosis was accurate when the following factors were included in the model: age, sex, education level, PI, number of missing teeth, previous oral hygiene instructions, and BMI (Table 1). Education level had the highest weight for the mathematical model (Figure 1).

Table 1- RSM analysis results involving different input variables to the output variable Definition 1 of periodontitis

Age	Sex	Education Level	Plaque Index (PI)	Number Of Missing Teeth Oral	Previous Hygiene Instructions	Body Mass Index (BMI)	Number Of Factors	Polynomial Degree	R^2 (Regression)	Maximum Absolute Error	Mean Absolute Error

Source: research data.

Figure 1 - Diagram with the relative weight of the input variables of the output variable Definition 1 of periodontitis (ZABOR *et al*., 2010), provided by SSAnova



Source: research data.

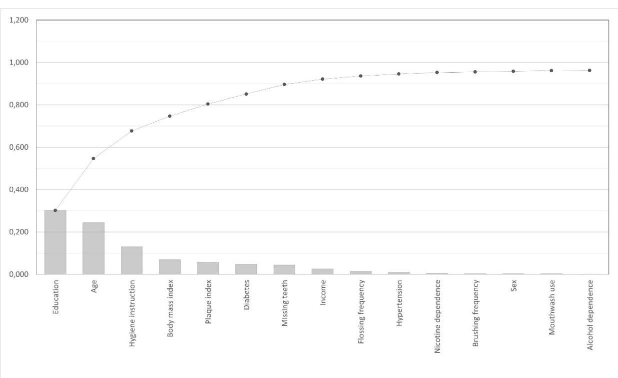
Case definition 2 was accurate for periodontitis diagnosis, including age, sex, education level, income, PI, previous oral hygiene instructions, frequency of brushing and type of toothbrush, and use of mouthwash in the model (Table 2). Education level had the highest weight for the mathematical model (Figure 2).

Table 2- RSM analysis results involving different input variables to the output variable Definition 2 of periodontitis

Age	Sex	Education Level	Income	Pi	Previous Oral Hygiene Instructions	Frequency Of Brushing	Type Of Toothbrush	Use Of Mouth Wash	Number Of Factors	Polynomial Degree	R^2 (Regression)	Maximum Absolute Error	Mean Absolute Error

Source: research data.

Figure 2 - Diagram with the relative weight of the input variables of the output Variable Definition 2 of periodontitis (Baelum & López 2012), provided by SSAnova



Source: research data.

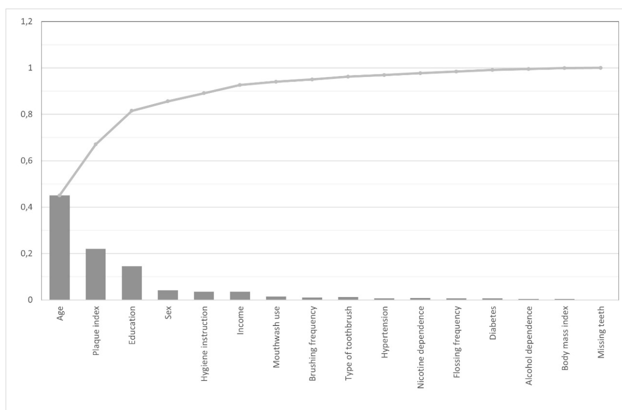
Case definition 3 showed accuracy for periodontitis diagnosis when including the following factors: age, education level, IP, number of missing teeth, previous oral hygiene instruction, BMI and diabetes (Table 3). The age had the highest weight for the mathematical model (Figure 3).

Table 3- RSM analysis results involving different input variables to the output variable Definition 3 of periodontitis

Age	Sex	Education Level	Plaque index (pi)	Number of missing teeth oral	Previous hygiene instructions	Body mass index (bmi)	Number of factors	Polynomial degree	R^2 (regression)	Maximum absolute error	Mean absolute error

Source: research data.

Figure 3 - Diagram with the relative weight of the input variables of the output Variable Definition 4 of periodontitis (EKE *et al*., 2012), provided by SSAnova.



Source: research data.

Using the RSM, this study developed and validated mathematical models for periodontitis diagnosis without periodontal probing evaluation. High representability for the three multifactorial mathematical models was observed. Therefore, the models could detect periodontitis based on demographic, socioeconomic, behavioral, systemic and local factors without requiring periodontal probing measurements.

The output variable of the model was the periodontitis case definition. There is no consensus regarding the best definition for periodontitis cases. A case definition system should facilitate identifying, treating, and preventing periodontitis in individual patients. Also, three components should be included: Identification of a patient as a periodontitis case;

Identification of the specific form of periodontitis; Description of the clinical presentation and other elements that affect clinical management, prognosis, and potentially broader influences on both oral and systemic health[19]. The present mathematical model aimed to identify periodontitis cases only. Focused on this goal, three widely used definitions of periodontitis cases were included in the models[11-13]. The new case definition by the EFP and AAP was not used as it does not stipulate a specific threshold of detectable CAL. Accordingly, due to clinical attachment level measurement errors with a standard periodontal probe, the case definition should be based on "detectable" interdental CAL[20].

The input variables analyzed (age, gender, education level, income, plaque index, number of missing teeth, nicotine and alcohol dependence, body mass index, hypertension, diabetes, brushing and flossing frequency, use of mouthwash, oral hygiene instruction) had different weigh in each of the models. However, education level, age and plaque index had the highest weight. According to previous studies, these three factors are strongly associated with periodontitis[21-23].

An advantage of this study was using the RSM, a consolidated method and widely used in different fields, comprising chemical industry, agriculture, pharmacy, environment, and mechanical engineering. Theirs advantages to analyze the interactive effects among different factors and the efficiency considerations have been encouraged research employed with these models. In addition, previous studies have reported the successfully adoption of RSM in the odontology research, whose purpose was to optimize the process parameters of dental materials[24-26] or testing anti-microbiological activities[27-29]. On other hand, limitations of the RMS include that the number of experiments increases with the number of independent variables, the poor prediction capability outside the experimental domain, and the model can tell what happens under different conditions but cannot explain the mechanism of the process[30]. The loss of 21.4% of samples because of missing information should be considered a limitation of this study. Ideally, the size of the population should be at least equal to the number of coefficients[31]. To overcome this limitation, mathematical interpolation techniques were applied. The interpolation technique used Levenberg-Marquardt's algorithm with a cycle of 20 pieces of training to find the best coefficient values. Although the presented results involving RSM have shown models with good representability, the limitations on sampling may have hindered the inference of variability of the process and, consequently, the adjustment of the models. Thus, future studies are necessary for validating the models and consolidating the RSM technique application for periodontitis case detection.

It is noteworthy that RSM may also be able to foresee and filtrate the variables with fundamental importance to be evaluated in extensive population studies. Therefore, optimizing time and resources in research and public health.

4 Conclusion

In conclusion, the multifactorial mathematical models developed could diagnose periodontitis according to different periodontitis case definitions using only variables with easy evaluation and non-invasive. Education level, age and plaque index variables presented the highest weight in the model. Further studies are necessary to validate these models in different populations.

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Authorship

DSPP, NSA and GNC contributed with Investigation, Data collect and Writing - original draft. PYN, JVLS and DTK contributed to the study design and the data analysis. PRC and JNS contributed to the project conception, administration and participated in all phases of the study. All authors revised and approved the final version of the manuscript.

References

1. Tran DT, Gay I, Du XL, Fu Y, Bebermeyer RD, Neumann AS, et al. Assessing periodontitis in populations: a systematic review of the validity of partial-mouth examination protocols. J Clin Periodontol 2013;40(12):1064-71. doi: https://doi.org/10.1111/jcpe.12165
2. AAP. American Academy of periodontology [internet]. Chicago: Diagnosis and examination. [cited 2022 Dec 14]. Available from: https://www.perio.org/research-science/periodontal-literature-review/diagnosis-and-examination/
3. Tsou MY, Liou JY, Ting CK, Lin SP. Patient response prediction with logistic regression in gastrointestinal endoscopy under midazolam-alfentanil sedation performed as well as response surface model. J Chinese Med Assoc 2018;81(12):1071-6. doi: https://doi.org/10.1016/j.jcma.2018.03.015
4. Chiou YW, Ting CK, Wang HY, Tsou MY, Chang WK. Enhanced recovery after surgery: Prediction for early extubation in video-assisted thoracic surgery using a response surface model in anesthesia. J Formos Med Assoc 2019;118(10):1450-7. doi: https://doi.org/10.1016/j.jfma.2019.07.024
5. Zhang D, Xiao N, Mahbubani KT, del Rio-Chanona EA, Slater NKH., Vassiliadis VS. Bioprocess modelling of biohydrogen production by Rhodopseudomonas palustris: Model development and effects of operating conditions on hydrogen yield and glycerol conversion efficiency. Chem Eng Sci 2015;130:68-78.
6. Bettinger D, Chinnici J. Utiliization of response surface modeling to evaluate the interaction between aflatoxin B1 and caffeine on egg adult viability in drosophila meganogaster. Va J Sci 1991;42(3).
7. Kong M, Lee JJ. A generalized response surface model with varying relative potency for assessing drug interaction.

Biometrics 2006;62(4):986-95. doi: https://doi.org/10.1111/j.1541-0420.2006.00579.x

8. Lee S Il. Drug interaction: Focusing on response surface models. Korean Journal of Anesthesiology. Korean J Anesthesiol 2010;58(5):421-34. doi: https://doi.org/10.4097/kjae.2010.58.5.421
9. Khuri AI, Mukhopadhyay S. Response surface methodology. Wiley Interdisciplinary Reviews: Computational Statistics. 2010.
10. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975;25(4):229-35.
11. Zabor EC, Klebanoff M, Yu K, Nansel T, Andrews W, Schwebke J, et al. Association between periodontal disease, bacterial vaginosis, and sexual risk behaviours. J Clin Periodontol 2010;37(10):888-93. doi: https://doi.org/10.1111/j.1600-051X.2010.01593.x
12. Baelum V, Lõpez R. Defining a periodontitis case: analysis of a never-treated adult population. J Clin Periodontol 2012;39(1):10-19. doi: https://doi.org/ 10.1111/j.1600-051X.2011.01812.x
13. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. J Periodontol 2012;83(12):1449-54. doi: https://doi.org/10.1902/jop.2012.110664
14. WHO. World Health Organization. Geneva: Obesity and Overwheight; 2021.
15. Torrungruang K, Tamsailom S, Rojanasomsith K, Sutdhibhisal S, Nisapakultorn K, Vanichjakvong O, et al. Risk indicators of periodontal disease in older Thai adults. J Periodontol 2005;76(4):558-65. doi: https://doi.org/10.1902/jop.2005.76.4.558.
16. AHA. American Heart Association [internet]. Dallas: Understanding Blood Pressure Readings; 2022
17. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 1991;86(9):1119-27. doi: https://doi.org/10.1111/j.1360-0443.1991.tb01879.x
18. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. J Stud Alcohol 1995;56(4):423-32. doi: https://doi.org/10.15288/jsa.1995.56.423
19. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH. Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol 2018;89:S173-S82. doi: https://doi.org/10.1002/JPER.17-0721
20. Tonetti MS, Greenwell H, Kornman KS. Staging and granding of periodontitis: Framework and proposal of a new classification and case definition. J Clin Periodontol 2018;89(Suppl 1):S159-S172. doi: https://doi.org/10.1002/JPER.18-0006. Erratum in: J Periodontol 2018;89(12):1475.
21. Boillot A, El Halabi B, Batty GD, Rangé H, Czernichow S, Bouchard P. Education as a predictor of chronic periodontitis: a systematic review with meta-analysis population-based studies. PLoS One 2011;6(7):e21508. doi: https://doi.org/10.1371/journal.pone.0021508
22. Pinto Filho JM, Araujo NS, dos Santos JN, Coelho TRC, Ramos Cury PR. The effect of dental plaque level and self-performed oral hygiene on periodontal status: a cross-sectional study in Brazilian Indigenous. J. Health Biol Sci 2021;9(1):1-6. doi: https://doi.org/10.12662/2317-3076jhbs.v9i1.3993
23. Ebersole JL, Graves CL, Gonzalez OA, Dawson D 3rd, Morford LA, Huja PE, Hartsfield JK Jr, Huja SS, Pandruvada S, Wallet SM. Aging, inflammation, immunity and periodontal disease. Periodontol 2000 2016;72(1):54-75. doi: https://doi.org/10.1111/prd.12135
24. Günhan B, Deliktaş D, Kaya G. Optimization of self-colored dental zirconia block parameters for an effective machining performance using response surface methodology based artificial bee colony. Dent Mater 2022;38(8):1248-60. doi: 10.1016/j.dental.2022.06.003.
25. Freitas JP, Agostinho Hernandez B, Gonçalves PJP, Baptista EC, Capello Sousa EA. Novel and simplified optimisation pathway using response surface and design of experiments methodologies for dental implants based on the stress of the cortical bone. Proc Inst Mech Eng H 2021;235(11):1297-309. doi: 10.1177/09544119211025375
26. Niroomand MR, Arabbeiki M. Statistical analysis of implant and thread parameters effects on dental implant stability and bone resorption using central composite design method. Proc Inst Mech Eng H 2019;233(12):1299-1309. doi: 10.1177/0954411919881250.
27. Kaewiad K, Nakpheng T, Srichana T. Dental floss impregnated with povidone-iodine coated with Eudragit L-100 as an antimicrobial delivery system against periodontal-associated pathogens. J Med Microbiol 2020;69(2):298-308. doi: 10.1099/jmm.0.001126.
28. Perić M, Rajković K, Milić Lemić A, Živković R, Arsić Arsenijević V. Development and validation of mathematical models for testing antifungal activity of different essential oils against Candida species. Arch Oral Biol 2019;98:258-64. doi: 10.1016/j.archoralbio.2018.11.029.
29. Lahiri D, Ray RR, Sarkar T, Upadhye VJ, Ghosh S, Pandit S, et al. Anti-biofilm efficacy of green-synthesized ZnO nanoparticles on oral biofilm: In vitro and in silico study. Front Microbiol 2022;13:939390. doi: 10.3389/fmicb.2022.939390
30. Nair AT, Makwana AR, Ahammed MM. The use of response surface methodology for modelling and analysis of water and wastewater treatment processes: a review. Water Sci Technol 2014;69(3):464-78. doi: https://doi.org/10.2166/wst.2013.733.
31. Kelley K, Maxwell SE. Sample size for multiple regression: obtaining regression coefficients that are accurate, not simply significant. Psychol Methods 2003;8(3):305-21. doi: https://doi.org/10.1037/1082-989X.8.3.305.