

Reproducibility of microhardness measurements in different surfaces

Reprodutibilidade de mensurações de microdureza em diferentes superfícies

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INTRODUCTION

Hardness tests are commonly used in the selection and specification of materials, researches and comparison of the

ABSTRACT

The aim of this study was to assess inter and intra-examiner reproducibility of Knoop hardness (KH) of two materials: composite resin (CR – Filtek™ Supreme XT- 3M ESPE) and bovine dental enamel (BDE). Five specimens were made for each material. The CR composite resin was inserted into a cylindrical metal matrix (4 x 2mm, ISO 4049) and photo-activated for 40s on the upper side (top) with a LED Celalux® (Voco, Germany). BDE specimens were taken from the buccal side of the teeth in blocks of 3x3x3mm that were embedded into acrylic discs, flattened with sandpapers and polished. Three KH readings were performed for each specimen using load of 25 gf applied for 5 seconds, at predetermined intervals: immediately and after 4 and 7 days of preparation. Each indentation was measured using a microhardness tester by three independent observers in each period of analysis. KH values were recorded separately by their respective observers. KH values were recorded separately by their respective observers. Data were statistically analyzed by ANOVA and Tukey's test ($\alpha=0.05$). To test for a possible correlation between measurements of each examiner, a linear regression analysis was performed. There was no statistical difference ($P> 0.05$) in intra and inter-examiner ratings, regardless of the period of analysis. A strong positive linear correlation (BDE- $r(\text{mean})=0.87$; CR- $r(\text{mean})=0.90$) was observed between KH measurements of the different examiners. The KH test showed high reproducibility among multiple examiners, regardless of periods or the materials used.

Keywords: Reproducibility of Results; Hardness Tests; Dentistry.

RESUMO

O objetivo deste trabalho foi estimar a reprodutibilidade intra e inter-examinadores das mensurações de dureza knoop (DK) de dois materiais: resina composta (RC- Filtek™ Supreme XT- 3M ESPE) e esmalte dental bovino (EDB). Cinco corpos-de-prova foram confeccionados para cada material. A RC foi inserida em matriz metálica circular (4 x 2 mm, ISO 4049) e fotoativada por 40 s na face superior com LED Celalux® (Voco, Alemanha). Para a confecção dos corpos-de-prova de EDB, blocos (3 x 3 x 3 mm) obtidos a partir da face vestibular de dentes bovinos foram incluídos em discos de acrílico, desgastados com lixa e polidos. Três leituras de DK foram realizadas com carga de 25 g durante 5 s para cada corpo-de-prova, em três tempos: imediatamente e após 4 e 7 dias da confecção. Cada indentação foi mensurada, em microdurômetro, por 3 observadores independentes, em cada período da análise. Os valores de DK foram registrados isoladamente por seus respectivos observadores. Os dados foram submetidos à ANOVA e Tukey ($\alpha=0,05$). Para avaliar o coeficiente de correlação (r) entre as observações de cada examinador, uma regressão linear foi realizada. Não houve diferença estatística ($P>0,05$) nas avaliações intra ou inter examinador, independentemente do período de análise. Observou-se forte correlação (EDB- $r(\text{médio})=0,87$; RC- $r(\text{médio})=0,90$) entre as mensurações de DK dos diferentes examinadores. O teste de DK apresentou elevada reprodutibilidade entre os múltiplos examinadores, independentemente dos períodos ou dos materiais utilizados.

Palavras-chave: Reprodutibilidade dos Testes; Testes de Dureza; Odontologia

performance of dental products and instruments. With regard to restorative dental materials, this property is important because it is directly related to tensile, shear, bending, abrasion, and also the degree of conversion, characteristics that

determine their clinical longevity^{1,2}. Besides directly influence the clinical longevity of restorative materials^{1,3}, the hardness may also indicate changes in dental structures^{4,5} when subjected to different conditions, substances or treatments.

By definition, hardness is the capacity of a material to resist penetration by hard edge, being directly proportional to its mechanical strength and wear resistance^{1,6}. It is a nondestructive test that can predict the distribution of the data properties of the material tested. Only the tests of Vickers and Knoop hardness evaluate this property in micrometer scale, since they use small and shallow area of the material (less than 19 μm), and therefore, are commonly used for analysis of dental materials, such as restorative composite resins¹. The Knoop hardness test has been widely used in dentistry research involving dental materials and dental mineralized tissues. The test is performed by means of a diamond indenter with pyramidal elongated shape which is pressed against the surface of the material, with predetermined load, thereby producing the indentation. The ratio of length, width and depth of impression is 30:4:1 and the relationship between the diagonals is 7:1. The larger diagonal produced is measured manually and the equipment automatically turns into a numerical value representing the hardness of the material^{1,6}.

For Knoop hardness test there occur large statistical fluctuations in the values obtained, which brings the need to check the interference factors. This occurs by the fact that, despite the simplicity of testing, the reliability of data is also related to the preparation of the specimens, which must be thoroughly flattened and polished; and also to the calibration of the operator to measure the larger diagonal of indentation, as this will determine the hardness value of the material tested. Any distortion in data collection may lead to impairment of research⁷. Thus, estimating the intra and inter-examiner reproducibility of measurements is of great value to the credibility of survey data from a study, and is given by the ability to obtain similar data in repeated indentations on the same surface.

Considering the increasing applicability of hardness test in several studies, due to its easy implementation is important to determine the reliability of data obtained. Thus, this study aims to estimate inter and intra-examiner reproducibility of data

obtained from a laboratory test of microhardness on the surfaces of composite resin and bovine dental enamel.

METHODS

Specimen preparation

To obtain the specimens of composite resin a cylindrical metallic mould (4 mm internal diameter and 2 mm thickness) was used. The mould does not allow the diffusion of light. The resin Filtek™ Supreme (3M ESPE, St. Paul, MN, USA) was packed to excess into the mould which was resting on a polyethylene sheet and a glass plate and then covered with a further polyethylene sheet and a glass slide. 1-kg weight (kilogram) was applied to set in order to expel excess material from the mould (Figure 1). Each specimen was photoactivated with LED CELALUX® (Voco, Germany) with a power density value of 776 mW cm^{-2} through the top glass slide for 40 s.

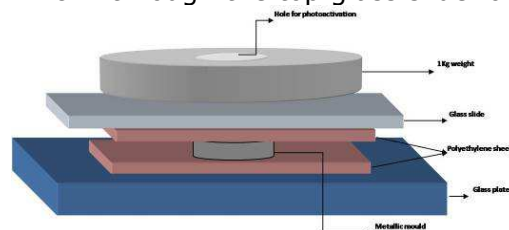


Figure 1 - Schematic drawing of the preparation of CR specimens.

For the preparation of bovine dental enamel specimens 10 freshly extracted upper central incisors were selected and stored in distilled water for seven days. Roots were removed and buccal enamel fragments were obtained from the crowns with a diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication. Each dental fragment (4 mm x 4 mm x 4 mm) was embedded in self-curing acrylic resin cylinders. Enamel surfaces were flattened with wet 600-, 1000- and 1200-grit aluminum oxide abrasive papers (3M ESPE, St. Paul, MN, USA) and polished with 3 and 1 μm -grit diamond pastes (Arotec S.A. Ind. e Com., Cotia, SP, Brazil) on a polishing machine (APL-4, Arotec S.A. Ind. e Com., Cotia, SP, Brazil). Figure 2 shows the scheme representing the preparation of BDE specimens.

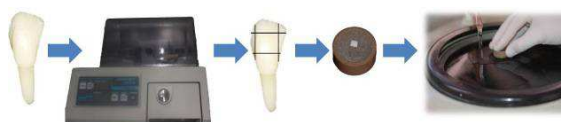


Figure 2 - Scheme representing the preparation of BDE specimens.

Hardness Test

Specimens were positioned perpendicularly to the long axis of the indenter to record the Knoop hardness number (KHN). A 25 g load Knoop indenter attached to a microhardness tester (Micromet 2100, Buehler, Lake Bluff, IL, EUA) performed three measurements during 5 seconds to determine the KHN of each specimen at each time interval (immediately and after 4 and 7 days of specimen preparation). The measurements were performed by three isolated examiners, which did not suffer any influence on the measurement and tabulation of data. Examiners received only handling instructions and calibration before the experiment. Mean values were statistically analyzed ANOVA and Tukey's test at the 5% level of significance. To test for a possible correlation between measurements of each examiner, a linear regression analysis was performed.

RESULTS

Intra-examiner agreement

Tables 1 and 2 present means and standard-deviations of the hardness measurements, as well as the intra-examiner comparison result for the three periods of analysis (initial, 4 and 7 days). No statistical difference was observed between the three different analysis times for both composite resin and bovine dental enamel surfaces.

Table 1 - KH means and standard-deviations (\pm) of composite resin at the different time intervals. Same letters, vertically, represent mean values similar to each other ($P>0.05$)

| | Examiner 1 | Examiner 2 | Examiner 3 |
|----------------|-------------------------------|-------------------------------|-------------------------------|
| 1st evaluation | 61.5 7 (2.14) ^A | 59.7 3 (1.94) ^A | 58. 56 (3.21) ^A |
| 2nd evaluation | 65.4 3 (3.24) ^A | 65.5 6 (4.11) ^A | 64. 59 (5.56) ^A |
| 3rd evaluation | 63.3 4 (4.31) ^A | 62.5 1 (4.42) ^A | 60. 75 (4.03) ^A |

Table 2 - KH means and standard-deviations (\pm) of bovine dental enamel at the different time intervals. Same letters, vertically, represent mean values similar to each other ($P>0.05$).

| | Examiner 1 | Examiner 2 | Examiner 3 |
|----------------|-------------------------|---------------------|---------------------|
| 1st evaluation | 264. 50 (17.93) A | 252.88 (10.10) A | 223.88 (33.10) A |
| 2nd evaluation | 245. 99 (13.62) A | 248.82 (13.56) A | 244.49 (11.98) A |
| 3rd evaluation | 239. 19 (65.12) A | 235.87 (59.99) A | 226.71 (61.24) A |

Inter-examiner agreement

Figures 3 and 4 show the inter-examiner correlations in the same analysis time. No statistically significant difference was found between the examiners and a strong correlation between the investigators independently of the material tested was observed ($r > 0.80$).

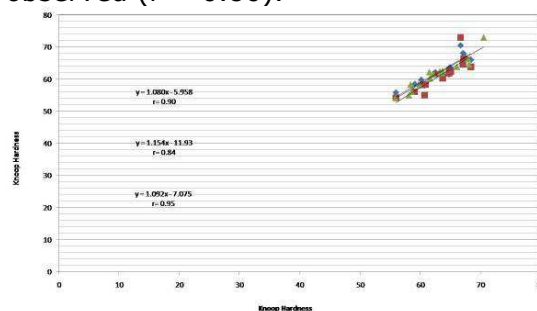


Figure 3 - Inter-examiner correlation for the KH results of the composite resin.

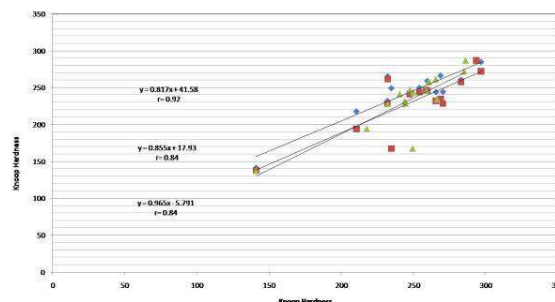


Figure 4 - Inter-examiner correlation for the KH results of the bovine dental enamel.

DISCUSSION

With constant technological evolution and consequent improvement of hardness testing machines, there is a growing trend of automation and reduced operator influence, allowing fully automatic measurements, ensuring accurate and reliable results. However, the reality of brazilian dental research must be

considered, since in the mechanical test laboratories of colleges and research institutes there are still present the digital hardness testers which require manual placement of the microscope and measurement of the indentation, bringing out the importance of measurements accuracy, which is directly influenced by the operator. The lack of attention to the validity of the research may lead to incorrect conclusions or results that are difficult to interpret⁸.

In a data collection must be considered on the one hand, the observer effect, and on the other, the effect of "observed object"⁹. Regarding the observer, it is an important source of error in the measurement of events in health area¹⁰, and this error can be quantified, considering that it can vary from person to person and also in the same individual from one occasion to another. Now, as the effect of the "observed object" for the hardness tests, special attention has to be given to the preparation of the specimens and to the proper planning of its surface since the Knoop indenter operates in this region. So any failure on the surface, both from roughness or imperfections arising from the preparation can involve the decrease of test accuracy, making it almost impossible the reproducibility. Radiologists and phthisiologists were the first to be interested in the magnitude of the errors, with investigations into the efficacy of radiographic techniques and equipments performed by the biostatistician Yerushalmy¹¹.

The concept of reproducibility, which is defined as "the consistency of results when the measurement or test is repeated"⁹, is critical in terms of information quality. Other terms used to express reproducibility are reliability and accuracy¹². Thus, a high degree of reliability and accuracy means to obtain similar results when the measurement is repeated.

In Dentistry, analysis of reproducibility has been made in order to verify the reliability of data obtained at different observation situations. Loffredo & Montandon¹³ conducted a preliminary analysis of the reproducibility of the CPITN index - Community Periodontal Index of Treatment Needs - finding 0.92 of intra-examiner agreement when the examiner has applied the same index on three different occasions. Other studies^{14,15} have reported the reproducibility of the Periodontal Index of Russell, finding 0.61 of correlation

between ratings given by two experts. It was concluded that this indicator is not conducive to reproducibility, because it does not provide ease of interpretation, considering the limit of 0.80 as the minimum value to be obtained for agreement¹⁶. The inter-examiner reproducibility found in this study has exceeded this limit, demonstrating a strong correlation between the different examiners for the Knoop hardness test.

As in the present paper, where information is measured numerically, ie, they are quantitative, a more satisfactory reproducibility result is expected in the situation where the standard-deviation is minimal, in other words where the observed results are close to the other. Table 1 shows high reproducibility of the test when the examiners performed measurements on the surface of composite resin, with no significant difference between the different times and a very small standard-deviation compared to the enamel surface. In Table 2 it is clear that high reproducibility is also obtained for dental enamel despite the finding of superior standard-deviations, which is probably due to greater resistance of the specimen to penetration of the diamond producing small indentations, thereby showing more expressive differences, however without compromising the results.

Other tests in health that generate numeric measures as this study are the readings of tuberculin test (Mantoux test). Ruffino-Netto et al.¹⁷⁻¹⁹ and Teruel et al.²⁰ analyzed the errors in these readings when different readers analyzed the same induration. They established as a necessary condition to participate in the work routine, an agreement of at least 0.80. The authors adopted a double-blind methodology for the reading of the induration diameter and emphasized the need for standardization of biological tests.

Despite the wide applicability of the hardness tests in the dental research area, literature shows few researches devoted to study its reproducibility. Given the scarcity of papers, this study aimed to compare measurements of hardness between different examiners, periods and surfaces evaluated. According to the results there was no statistically significant difference between the measurements performed by one investigator, as well as among those carried out by different examiners independently of the evaluated surface.

Respecting other factors such as the appropriate preparation of the specimens and calibration of examiners, the Knoop hardness test showed to be reproducible. Thus, a microhardness experiment can be performed in different periods and by different operators, whereas they are calibrated and perform measurements on the same equipment. Moreover, it is recommended that whenever repeated measurements is performed by the same operator or by different operators, it should be performed a pilot test considering the agreement of at least 0.80 prior to the start of the final experiment.

CONCLUSION

- The Knoop hardness test showed high reproducibility between multiple examiners, regardless of the periods and the surface tested;
- High accuracy is obtained when using the same observer and evaluation period. The reproducibility of the KH measurements was equivalent in bovine dental enamel and composite resin surfaces.

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