UNIVERSIDADE FEDERAL DE UBERLÂNDIA

FACULDADE DE ODONTOLOGIA

VITOR CARDOSO COSTA

AVALIAÇÃO DA MICROTOMOGRAFIA COMPUTADORIZADA DO REPARO ÓSSEO ALVEOLAR: QUAL REGIÃO VOCÊ DEVE ANALISAR?

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AVALIAÇÃO DA MICROTOMOGRAFIA COMPUTADORIZADA DO REPARO ÓSSEO ALVEOLAR: QUAL REGIÃO VOCÊ DEVE ANALISAR?

Trabalho de conclusão de curso apresentado a Faculdade de Odontologia da Universidade Federal de Uberlândia, como requisito parcial para obtenção do título de Cirurgião-dentista.

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UBERLÂNDIA

2021

ABSTRACT

Objeticve: Evaluate the influence of the extraction socket (distal or lingual root) and the type of region of interest (ROI) definition (manual or predefined) on the assessment of alveolar repair after tooth extraction using micro-computed tomography (micro-CT).

Materials and Methods: The sample consisted of micro-CT volumes of the mandibles submitted to extraction of the mandibular right first molar (M1) of seven Wistar rats. The reconstructed images were analyzed using the extraction sockets: D- distal and IL- intermediate lingual root; and the ROI: MA- manual, CR- central round and PR- peripheral round. The BV/TV values obtained were analyzed by ANOVA two-way with Tukey post hoc test (α = 5%).

Results: In relation to the analyzed extraction socket, the D socket resulted in significantly lower BV/TV values than the IL socket, for the groups MA (P = 0.001), CR (P <0.001), PR (P <0.001). For the region of interest (ROI), BV/TV was significantly higher (P <0.001) for the MA group (75.11 ± 6.69) compared to CR (65.31 ± 5.16) and PR groups, with lower BV/TV for CR (55.96 ± 7.35) in the D socket. However, no significant difference was observed for the groups MA (91.38 ± 4.32, P = 0.855), CR (92.12 ± 5.49, P= 0.769) and PR (93.08 ± 4.16, P = 0.453) in the IL extraction socket.

Conclusion: Different ROIs definitions and the extraction socket being analyzed affect the morphometric results in micro-CT. The predefined method with standardized ROI in the central region of the bone defect in the distal extraction socket resulted in a more effective assessment of bone volume demonstrating the most critical region of the bone neoformation process.

keyword: micro-computed tomography, imaging, animal models, alveolar bone, tooth extraction

SUMÁRIO

1 INTRODUCTION

The alveolar bone is characterized by a continuous and rapid remodeling in response to stimuli [1,2,3]. Several clinical procedures, such as rehabilitative therapies, depend on the socket-healing process after tooth extractions and your understanding in preclinical studies is crucial. Therefore, the characterization of the dynamic process of bone to replace an extracted tooth and improvement of alveolar bone regenerative strategies is a topic of special interest in Dentistry [4,5,6].

The Micro–Computed Tomography (Micro-CT) quickly become a standard tool in the assessment of bone microarchitecture in animal models, as the complimentary alternative the traditional histomorphometry [7,8,9,10,11,12]. The nondestructive method, short turnaround time, volumetric analyses are appealing in the analysis of the microarchitecture of native and newly formed bone by means of morphometric parameters (2D and 3D) and mineral density [7,9,13,14]. In addition, the same sample can later be prepared for histologic analysis [7]. As Micro-CT use has expanded, analyses have grown to encompass complex structures as in the dentoalveolar complex, and has been widely used to study alveolar bone remodeling [12,15,16,17]. However, some variables and selected parameters in Micro-CT may affect the morphological outcomes[18].

One analysis parameter that influences on the quality of the results is the use the ROIs, which should be delimited based on experimental questions to be answered and it should be used with caution as it can bias results and provide imprecise values [12,19,20]. This process could be done manually [15,21] or in an automatic/semi-automatic fashion [11,22,23]. Different formats of ROIs (rectangular, polygonal, round, cubic, ring)

can be used as a representative sample in bone neoformation studies [12,24]. Another factor taken into account in alveolar repair analysis is the location of the ROI, i.e., the extraction socket used for analysis. Studies have evaluated the distal extraction socket [21,24], the mesial extraction socket [23,25], nonetheless, densities of the dentoalveolar tissues and the amount of neoformed bone vary by location and the size of the socket, which makes it difficult to direct compare with other models and studies [12,24].

Despite current advances, there is no standardization of parameters for analyzing Micro-CT images in preclinical studies. Related to protocols for rodent jawbones, the literature is particularly scarce and the methodology for a reproducible quantitative analysis is not informative or detailed enough, what makes it difficult to understand how analyses were performed and if findings can be broadly compared to other models and studies [12,24], which may compromise the scientific impact of the studies [20]. We began this study with the following question: Micro- CT evaluation of alveolar bone repair: what region should you analyse? Thus, the aim of this study was to evaluate the influence of the extraction socket (distal or lingual root) and the type of region of interest (ROI) definition (manual or predefined) on the assessment of alveolar repair after tooth extraction using micro-CT, and understand the effect of variation in these parameters and which method can result in more effective assessment of bone volume. The null hypothesis was that the type of ROI and the analysis region did not influence the outcome from Micro-CT analysis.

2 MATERIALS AND METHODS

This study was carried out in compliance which conform to ARRIVE guidelines for preclinical studies and the normative guidelines of the National Council for Animal Control and Experimentation (CONCEA). The sample consisted of micro-CT images of the mandible of 7 male Wistar rats that were acquired for previously approved research by the Research Ethics Committee of the institution (CEUA protocol 013/19).

Mandibular right first molar (M1) extractions were performed using luxators hollemback Sculptor No.3 (Golgran, São Paulo, São Paulo, Brasil) and micro-forceps (n°5, Golgran, São Paulo, São Paulo, Brasil) to avoid fracture of the roots following the established protocols [5]. The animals were euthanized 14 days after tooth extraction by intraperitoneal overdose of thiopental (150 mg/kg). The hemimandibles were fixed in 4% paraformaldehyde solution and 10% 0.1 M phosphate buffer (pH 7.4) during 48h and scanned using X-ray microcomputed tomography Skyscan 1176 (Bruker, Kontich, Belgium) using the following settings: isotropic voxel size of 9 µm (65 kV; 385 µA); aluminum filter of 1 mm; rotation step of 0.5º; 3 frames per rotation degree; and 180º rotation, scan time 55 minutes. Each mandible was fixed with wax on the scanning platform to ensure a standardized position with the long axis vertical to the horizontal plane wrapped in wet paper to maintain moisture during the scanning procedure. After scanning, the images were imported into NRecon Reconstruction software (version 1.6.6.0, Bruker, Kontich, Belgium) for reconstruction in grayscale, presenting x-ray attenuation coefficients with values related to bone structure. The images were reconstructed using the following settings: 40% for beam hardening correction, 0 for

smoothing and 12 to reduce ring artifacts. The reconstructions included the mandibular first and second molar region.

Data Viewer software (SkyScan) was used to adjust the images of the hemi-mandibles to standard positioning in order to get better positioning of the distal and lingual tooth socket of the extracted mandibular first molar. The images in the axial/ transverse plane with standard orientation were exported to CTAn software (version 1.14.4.1, SkyScan, Bruker, Belgium) and only the sections including the distal and lingual extraction socket of the extracted mandibular first molar was selected to delineate the ROI showing alveolar socket healing, being a total of 80 slices along the cervical, middle and apical third of the socket for all samples. The reconstructed images were analyzed using the extraction sockets: D- distal and IL- intermediate lingual root; and the ROI: MA- manual, CR- central round and PR- peripheral round. The segmentation of region of interest in the D and IL extraction socket was performed individually for each ROI format. In the MA group, a personalized ROI was acquired manually using the computer mouse to delimit the extraction socket, respecting its contours. In the CR group, a predefined ROI of round shape of 0.5 mm in diameter was selected and centrally positioned in the extraction socket of the mandibular right first molar (M1). In the same manner, for the analysis of PR group, a standardized ROI of round format of 0.5 mm in diameter was positioned in the region close to the buccal surface in the extraction socket of the M1 (Fig.1). The entire region was defined by interpolation of the ROIs every 10 slices. After ROI delimitation, a global threshold ranging from 71-255 was established. The threshold was defined as the mean of the automatic threshold values (Otsu method) calculated from 10 samples from a previous study. A single operator carried out all the analysis. Then, three-dimensional analysis

of the region of interest was performed to examine the features of the bone microarchitecture, according to Bouxsein *et al*. 2010 [7]: percent bone volume (BV/TV,%).

2.1 Statistical analysis

Statistical analysis was performed using SigmaPlot® (SigmaPlot v13.1; Systat Software Inc.). The influence of extraction socket (distal or lingual root) and the type of region of interest (ROI) definition (manual or predefined) on BV/TV values was assessed using two-way analysis of variance (ANOVA) with Tukey's post hoc test, considering a significance level of $\alpha=0.05$.

3 RESULTS

The BV/TV means and standard deviations are described in Table 1. Two-way ANOVA showed that BV/TV values were significantly influenced by type of ROI ($P < 0.001$), analysis region ($P < 0.001$), and the interaction between these factors ($P < 0.001$).

In relation to the analyzed extraction socket, the D socket resulted in significantly lower BV/TV values than the IL socket, for the groups MA ($P = 0.001$), CR ($P <0.001$), PR ($P <0.001$). For the region of interest (ROI), BV/TV was significantly higher ($P <0.001$) for the MA group (75.11 ± 6.69) compared to CR (65.31 ± 5.16) and PR groups, with lower BV/TV for CR (55.96 ± 7.35) in the D socket. However, no significant difference was observed for the groups MA (91.38 ± 4.32, $P = 0.855$), CR (92.12 ± 5.49, $P= 0.769$) and PR (93.08 ± 4.16, $P = 0.453$) in the IL extraction socket.

4 DISCUSSION

The parameters used in the analysis in Micro-CT may have a direct influence on the final characteristics of the image [26] and, consequently, in the morphometric outcome [20]. This study evaluated the influence of analysis parameters, including the region of interest (ROI) and the extraction socket of Micro-CT images on the evaluation of alveolar bone repair after tooth extraction the M1 in rats. The amount of neoformed bone (BV/TV) were significantly influenced by the ROI shape, the evaluated extraction socket, and the interaction between these factors. Thus, the null hypothesis that these variables would not influence the outcome from Micro-CT analysis was rejected.

Several preclinical models have been used to evaluate bone repair process in surgically created defects, such as femur/tibia [27,28,29,30], and extraction socket in mandibles [21,23, 25,31,32]. The literature is conflicting regarding the portion of extraction sockets selected for the ROI. Alveolar repair has been analyzed in different extraction sockets (e.g., extraction socket of the distal [21,24] and mesial [23, 25]. Moreover, different ROIs formats are found in the literature, including those manually delineated [15,21] and predefined shapes [11,22,33]. However, the method used for ROI delimitation can affect the morphometric results in Micro-CT [20,34]. Besides, it has been demonstrated that by changing the position of the ROI, a variation of 12%–37% in the BV value was observed [35]. Thus, the direct comparison of results among the studies is not recommended.

In our study, a rounded standardized shape for determination of the ROI was evaluated and changes in its position resulted in significant differences for BV/TV values. Less bone neoformation for the central round ROI was detected in comparison to the peripheral ROI. In addition, higher BV/TV values were observed for manual ROI

for distal root M1. The bone healing occurs as a centripetal process, as a result of bone formation originating on the socket walls and subsequently confluent until filling all the socket extension [33,36,37]. This process explains the results found in the present study, demonstrating a critical area of bone repair in the central region of the socket, with the presence of a hypomineralized bone (osteoid) [38]. Additionally, ROIs delineated manually to quantify the bone neoformation process may include regions that are not part of the region of interest (e.g., cortical bone or cancellous bone outside the extraction socket) since the healing area might not be well defined. Thus, inaccurate BV/TV values may have been obtained with this method. On the other hand, standardized ROIs used as a representative sample can avoid biased results in the evaluation of bone repair [6,12,24], taking into account that the ROI must be chosen stringently and consistently across samples to minimize selection bias [12]. Thus, the use of standardized ROIs compatible with the critical region of the socket throughout analyses is strongly recommended.

Although the bone neoformation process in extraction socket is a well-known process [39,40], large bone defects (critical defect) represent major problems on the bone-healing process [41], being bone grafts necessary for reconstruction. In the present study, the BV/TV values were not affected by the ROI format for the IL socket. Moreover, a significant higher amount of new bone formation was observed in the IL socket compared to the D socket, regardless of the ROI shape, demonstrating more bone formation with almost complete socket healing. This result was expected because of the difference in the root diameter. IL sockets are of smaller size and consequently leads to earlier socket healing [41].

It is important to previously determine all the crucial parameters to answer the purpose of the study [9]. Another important aspect of the micro-CT analysis that must be taken into consideration is the size of the socket (i.e., the root to be analyzed). Studies that aim the evaluation of therapies to increase bone quality or quantity [42], or in the evaluation of systemic conditions such as radiotherapy [30,43], diabetes [29] and osteoporosis [17], the evaluation of larger diameter sockets is recommended for being more critical, so the effect of such therapies or conditions can be properly evaluated.

The majority of the experimental reports lack details of the parameters applied for micro-CT image acquisition and analysis [24,44]. Regarding the ROI definition, few studies have clearly reported how this parameter was set. The method of the study should provide descriptions of the size and location of the ROI since the results can be affected by those parameters. Furthermore, it may be useful to provide a figure of a representative sample to illustrate the selected ROI and demonstrate the region being analyzed [7,12.]

Regarding the experimental period, extraction socket repair is completed in 28 days in healthy rats [6,45,46]. Our study is in accordance with the period of the normal course of alveolar bone repair in rats after tooth extraction, with the bone formation phase observed at 14 days.

5 CONCLUSION

Within the limitations of this preclinical study, the following conclusions can be drawn:

1. Different ROIs shapes and positions within the extraction socket affect the morphometric results in micro-CT.

2. Bone neoformation outcome (BV/TV) for alveolar bone repair after tooth extraction were significantly influenced by the ROI (manual drawn or predefined shape) and the extraction socket (distal or lingual root).

3. The predefined method with standardized ROI in the central region of the bone defect in the distal extraction socket resulted in a more effective assessment of bone volume demonstrating the most critical region of the bone neoformation process.

Acknowledgments

The authors are grateful to Rede de Biotérios de Roedores da Universidade Federal de Uberlândia. This study was supported by grants from the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Conflict of Interest

The authors declare that they have no conflict of interest.

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7 TABLES

Table 1. Mean and standard deviation of BV/TV (%) values of the bone morphometric parameters analyzed in the alveolar repair area by micro-CT.

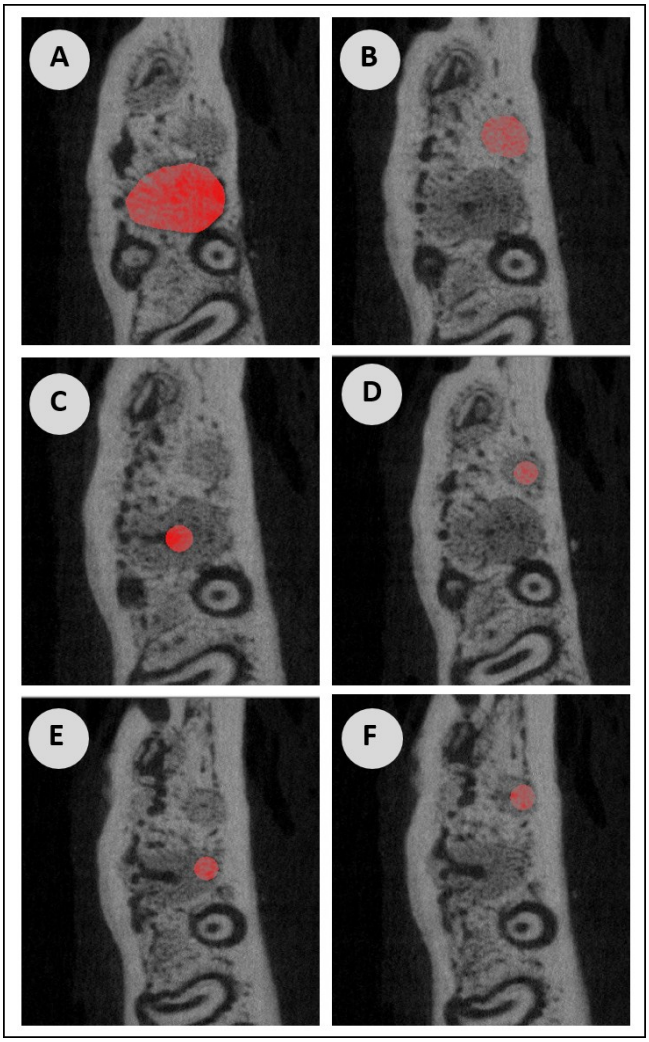
Region of interest / Extraction socket	**Manual ROI**	**Central round ROI**	**peripheral round ROI**
Distal M1	75.11 ± 6.69^{Ab}	55.96 ± 7.35^{Cb}	65.31 ± 5.16^{Bb}
Intermediate lingual M1	91.38 ± 4.32^{Aa}	92.12 ± 5.49^{Aa}	93.08 ± 4.16^{Aa}

Different letters indicate statistically significant differences verified by Tukey test ($p<0.05$). Uppercase letters are used for comparing region of interest (manual, central round, peripheral round), lowercase letters are used for comparing extraction socket (distal and intermediate lingual root of the lower first molar (M1).

Figure caption

Fig. 1. Demonstration of the region of interest (ROI) delimitation in the analyzed extraction socket. A) manual ROI and distal extraction socket of the lower first molar (M1). B) manual ROI and intermediate lingual extraction socket of the M1. C) central round and distal extraction socket of the M1. D) central round and intermediate lingual extraction socket of the M1. E) peripheral round and distal extraction socket of the M1. F) peripheral round and intermediate lingual extraction socket of the M1.

Figure 1



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Declaration of funding source and conflicts of interest

Authors always state the source of any funding for research or towards publication of their article, including the government and private or commercial sources.

Conflicts of interest is required to be stated in the manuscript. Conflicts of interest are the interest of the author(s) which may influence the objectivity, integrity and value of the publication. The examples are; Employment by any organization with a financial interest in or financial conflict with the publication of the article. Financial support by any organization with a financial interest in or financial conflict with the publication of the article. Personal financial interests: financial interests such as stocks and shares in companies with a financial interest in or financial conflict with the publication of the article; consultation fees or other remuneration from organizations with a financial interest in or financial conflict with the publication of the article; patents or patent applications which may be affected by publication of the article. Membership to organizations with a financial interest in or financial conflict with the publication of the article.

If a reviewer or editor of a manuscript feels a conflict of interest in making a decision on a manuscript, (s)he should return the manuscript to the editorial office.

Statement of Informed Consent and Institute Review Board

Studies on human subjects must have been approved by the Institutional Review Board (IRB). Also, informed consent must be obtained from the patients who participated in the study. The manuscript must include a statement of the informed consent and ethical approval including IRB information in Materials and Methods. These documents can be requested from the editor, reviewer, or publisher. In case of animal study, authors should indicate whether institutional and national guides for the care and use of laboratory animals were followed.

Authorship

All authors should have made substantial contributions to all of the following according to the guidelines of the International Committee of Medical Journal Editors (ICMJE): 1. "Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work"; AND 2. "Drafting the work or revising it critically for important intellectual content"; AND, 3. "Final approval of the version to be published"; AND 4. "Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved". All authors must participate in determining the order of authorship.

Originality, Duplicate Publication, and Secondary Publication

The submitted manuscript should be original and it should not be considered to be published to other journals at the same time. Any part of the manuscript such as figures and tables should not be duplicated in any other published article without permission of the Editorial board. If duplication is found, the authors might be imposed penalty according to the decision by our research ethics committee. Manuscript can be republished if it satisfies the conditions of secondary publication of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

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The Editor reserves the right to make changes which may clarify or condense papers where this is considered desirable.

SUBMISSION OF MANUSCRIPTS

The manuscript is requested to be submitted to the editor-in-chief electronically by online at http://www.isdent.org. For online submission, authors prepare the following electronic files, 1) Cover letter, 2) Manuscript, and 3) Figures. Authors don’t have to submit the copyright transfer form during the manuscript submission. The publisher will ask the form to the authors only for the accepted manuscript.

Cover letter

•Cover letter should be written by using MS-Word file in electronic form.

•This should contain the title of article, authors’ full names, position, and institutional affiliation(s). If several authors and institutions are listed, they should be clearly indicated with which department and institution each author is affiliated with superscripts as “1”, “2” ..., and etc.

•In a separate paragraph, address for correspondence, including the corresponding author''s position and name, full address (institutional affiliation, city, zip-code and country), telephone number, and email address, should be given.

•Information concerning the sources of financial support should be placed as a footnote.

•All authors' ORCID IDs are required to be listed at the last of Cover letter.

Manuscript

•Manuscript should be written by using MS-Word file in electronic form and must be typed in double space on A4 size paper with a 3 cm margin of every side.

•All manuscript pages are to be numbered consecutively.

•Neither the author's names nor their affiliations should appear on the manuscript pages.

•Manuscript should be organized in the following order: Title Page (title only without anything else), Abstract, main body of manuscript, Acknowledgement, References, Tables, Figure legends. Each section should begin on a new page. The details are in “Guidelines for Each Type of Manuscript” as below.

•Any equipment and drugs mentioned in the manuscript should specify the manufacturer and their locations (city and country) in the parentheses.

•Radiation and Measurements should be in accordance with the International System of Units (SI). Refer “SI units in radiation protection and measurements. NCRP Report No. 82.” and “Lundberg GD, Iverson C, Radulescu G. Now read this: the SI units are here. JAMA 1986; 255: 2329-39.”

•Once accepted, the final version of the manuscript can be submitted by email.

Figures

•Figures should be submitted as BMP/TIFF/JPG format original files over 300 dpi for color or radiographic images and over 1200 dpi for line drawings. Submission of the edited figures should be avoided. Inadequate figures can be the reason of reject.

•Each figure file should be named as the figure number and alphabet (i.e.: Fig. 1, Fig. 2, Fig. 1A, Fig. 1B, etc)

•Do not include the personal information on the figure or radiograph. An individual should not be recognizable in the photographs unless written consent of the subject has been obtained and is provided at the time of submission.

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The processing is informed to the corresponding author. Please review the check lists just before submission. Submission of a manuscript to ISD is free. Regarding the printed journal and reprints, contact the Editor by email please.

Editor-in-chief

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GUIDELINES FOR EACH TYPE OF MANUSCRIPT

Original Articles

1)**Abstract**

•This should not exceed 250 words and should be provided on a separate page.

•The abstract should be constructed under the following subheadings: Purposes, Materials and Methods, Results, and Conclusion.

•Describe each item separately in the following order.

Purpose: In one or two sentences, indicate the specific purpose of the article, and indicate why it is worthy of attention. The purpose stated here must be identical to the one given in the title of the paper and the introduction.

Materials and Methods: Describe succinctly the methods used to achieve the purpose explained in the first paragraph, stating what was done and how, how bias was controlled, what data were collected, and how the data were analyzed.

Results: The findings of the methods described in the preceding paragraph are to be presented, with specific data. All results should flow logically from the methods described.

Conclusion: In one or two sentences, state the conclusion of the study. This must be related directly to the purpose of the papers, as defined in the first paragraph of the abstract.

•Do not use abbreviations or reference citations

•At the bottom of the abstract, select up to 4 key words from the current Medical Subject Headings (MeSH) in Index Medicus. Refer the website "http://www.nlm.nih.gov/mesh/MBrowser.html".

2)**Introduction**

Briefly describe the purpose of the investigation, including relevant background information.

3)**Materials and Methods**

Describe the research plan, the materials (or subjects), and the methods used, in that order. When experimental methodology is the main issue of the paper, describe the process in detail so as to recreate the experiment as closely as possible. The statements for IRB and informed consent should be described in Material and Methods.

4)**Results**

Present these in a clear, logical sequence. Since biometrics involves variations in exact measurements, follow the rule of using statistics when experimentation is described. If tables are used, do not duplicate tabular data in the text, but do describe important trends and points.

5)**Discussion**

Observations pertaining to the results of research and other related materials should be interpreted for your readers. Emphasize new and important observations; do not merely repeat the contents of the results. Explain the meaning of the observed opinion along with its limits, and within the limits of the research results connect the conclusion to the purpose of the research. In a concluding paragraph, summarize the result and its meaning.

6)**References**

•Start on a separate page, numbering the references consecutively in the order in which they appear in the text.

•All references should be cited in the text, and in the text indicate the reference with superscription with Arabic numerals.

•Journal titles should be abbreviated according to the Index Medicus.

•All authors are to be listed when six or fewer; when there are seven or more, the first six should be given, followed by 'et al'.

•After writing the authors' last names first, the initial of their first and middle names should be capitalized.

•For all references, the starting page and the last page numbers are to be given.

•The formats are illustrated in the following examples.

Journal article:

Hayakawa Y, Eraso FE, Scarfe WC, Farman AG, Nishidawa K, Kuroyanagi K, et al. Modulation transfer function analysis of a newly revised rotational panoramic machine. Dentomaxillofac Radiol 1996; 25: 32-6.

Complete book:

Goaz PW, White SC. Oral radiology; principles and interpretation. 3rd ed. St. Louis: Mosby-Year Book Inc; 1994.

Chapter in the book:

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

Journal article in press:

Figueiredo PT, Leite AF, Freitas AC, Nascimento LA, Cavalcanti MG, Melo NS, et al. Comparison between computed tomography and clinical evaluation in tumour/node stage and follow-up of oral cavity and oropharyngeal cancer. Dentomaxillofac Radiol (in press).

Abstracts:

Mileman PA, Espelid I. Radiographic treatment decisions - a comparison between Dutch and Norwegian practitioners. J Dent Res 1986; 65: 609 (Abstr 32).

Letter to the editor:

Farman AG. Panoramic radiographic images and the prediction of asymmetry. Dentomaxillofac Radiol 2006; 35: 129 (letter).

Others:

Follow the form indicated in Uniform Requirements (1997).

7)Figure Legends

•Numbering the figures consecutively in the order in which they appear in the text.

•Describe the description using one complete sentence rather than a phrase or clause.

•All abbreviations should be described as full words in each figure.

•Representations of microscopic images should include the magnifying power.

8)Tables

•Tables should be positioned at the last of manuscript. If it would be impossible to be included in the manuscript, authors can provide the tables in a separate MS-Word file.

•For tables, Arabic numerals should be employed. The title of the table should be clearly stated in the form of a phrase or clause.

•Tables are recommended not to be longer than one page and must contain at least four lines and two columns of data.

•Tables are to be numbered in the order in which they are cited in the text.

•Abbreviations should be defined in an explanatory note below each table.

•Use of the symbols, follow the sequence: *, †, ‡, §, ‖, ¶, **, ††, ‡‡

•Tables should be self-explanatory and readily comprehensible.

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Universidade Federal de Uberlândia

– Comissão de Ética na Utilização de Animais –

CERTIFICADO

Certificamos que o projeto intitulado "Efeito da radioterapia na progressão do reparo ósseo e de sítios pós-extração dentária", protocolo nº 013/19, sob a responsabilidade de **Priscilla Barbosa Ferreira Soares** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADA** pela COMISSÃO DE ÉTICA NA UTILIZAÇÃO DE ANIMAIS (CEUA) da UNIVERSIDADE FEDERAL DE UBERLÂNDIA, em reunião **10 de maio de 2019.**

(We certify that the project entitled "Efeito da radioterapia na progressão do reparo ósseo e de sítios pós-extração dentária", protocol 013/19, under the responsibility of Priscilla Barbosa Ferreira Soares - involving the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata, for purposes of scientific research - is in accordance with the provisions of Law nº 11.794, of October 8th, 2008, of Decree nº 6.899 of July 15th, 2009, and the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and it was approved for ETHICS COMMISSION ON ANIMAL USE (CEUA) from FEDERAL UNIVERSITY OF UBERLÂNDIA, in meeting of May 10th, 2019).

Vigência do Projeto	Início: 01/07/2019 Término: 01/11/2022
Espécie / Linhagem / Grupos Taxonômicos	Rato heterogenico Wistar
Número de animais	112
Peso / Idade	300g/ 90 dias
Sexo	Macho
Origem / Local	Rede de Biotérios de Roedores da UFU – REBIR-UFU
Local onde serão mantidos os animais:	Rede de Biotérios de Roedores da UFU – REBIR-UFU

Uberlândia, 04 de Junho de 2019.

Prof. Dr. Lúcio Vilela Carneiro Girão
UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Comissão de Ética na Utilização de Animais /UFU
Coordenador da CEUA
Portaria Nº 542 DE 10 DE MAIO DE 2019