RESEARCH ARTICLE

Evaluation of soft tissues simulant materials in cone beam computed tomography

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Objectives: To evaluate different materials in simulating soft tissues and to analyze the influence of these materials on the mean (MPIV) and standard deviation of pixel intensity values comparing them to a gold-standard in CBCT images.

Methods: Images of three piglet heads with their soft tissues intact (gold-standard) and different simulant materials were acquired: ice, modelling wax, and ballistic gelatin, with the same thickness of the original soft tissues. The pixel intensities were measured in dental, bone and soft tissues regions, in the mandible and maxilla, for all the groups. Analysis of variance, Dunnett's, Pearson's and linear regression tests were performed.

Results: The simulators did not significantly change the MPIV of teeth in comparison with the gold-standard ($p = 0.1017$). Only ice ($p = 0.0156$) affected the MPIV of bone. Wax ($p = 0.001$) and ice ($p = 0.0076$), but not ballistic gelatin ($p = 0.5814$), altered the MPIV of soft tissue regions. When assessing the influence of the location (mandible or maxilla) among the simulants, the differences were significant only for the soft tissue regions. Standard deviation was not influenced by simulants ($p > 0.05$), but ballistic gelatin presented the lower variability.

Conclusions: The ballistic gelatin was the best soft tissue simulant since it had the lowest influence on the pixel intensity values for all regions.

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Introduction

Cone beam CT (CBCT) has largely contributed to diagnosis and treatment in dentistry. In CBCT, the X-ray photon attenuation is represented by multiple values (pixel intensity) assigned to each greyscale representation of the image.¹ Denser tissues (teeth and bones) absorb more radiation than the soft ones, producing hyperdense and hypodense images, respectively. Due to the limitations of how grey values are represented in CBCT and the technological advances in equipment and software, an increase in the number of studies using this image modality has been observed.²

Each voxel expresses an intensity relative to the linear attenuation coefficient of the structures crossed by the radiation in tomographic images. Different from those in multidetector CT, where the density can be assessed using the Hounsfield units, the values in CBCT are not a definitive representation of the physical characteristics of the material.^{1,3} Several studies sought to understand the limitations in determining voxel values in CBCT and concluded that they are affected by different types of artefacts, such as those caused by secondary radiation, resulting from the interaction of the X-ray beam with matter in association with a flat image detector. Other factors such as reduced milliamperage, which produces

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greater image noise, and computational factors of the reconstruction process may also affect.^{4,5}

Due to the biological risks inherent to ionizing radiation, many studies on CBCT are conducted *in vitro* and *ex vivo*. These studies have used dry anatomical specimens and radiographic phantoms, which often require a soft tissue simulant material to reproduce the interactions that occur during the radiographic acquisition.^{6,7} Soft tissue simulation is important in several areas of dentistry since it brings additional information to diagnosis and planning.²

Two-dimensional imaging studies have suggested the use of wax and water as soft tissue simulant materials.^{6–11} However, none of these studies used a gold standard to evaluate these materials at the same thickness as that of the original tissue. Thus, these results can be questioned, and another methodology can be proposed to find materials that better simulate soft tissue and most closely resemble the clinical reality in oral radiology. Therefore, the present study used a gold standard and evaluated other materials such as ice, modelling wax and ballistic gelatin.

The present study aimed to evaluate different materials in simulating soft tissues and their influence on the pixel intensities, comparing them to a gold standard in CBCT images.

Methods and materials

This study was exempted from review by the Ethics Committee for Animal Research of Biology Institute—UNICAMP.

Sample

The sample was composed by three fresh piglet heads, which, as inclusion criteria, had intact surrounding soft tissues and preserved maxillomandibular anatomical structures. These heads were used as the gold-standard and had their soft tissues replaced with the three simulant materials: ice, modelling wax (Lysanda Ltd, São Paulo, SP, Brazil), and ballistic gelatin. Briefly, ballistic gelatin was made by mixing 48 g of unflavoured gelatin, 200 ml of glycerine, and 500 ml of water. The mixed ingredients were boiled for 25 min and left to rest for 20 min before use.

Image acquisition

The CBCT volumes were acquired in a Picasso Trio (Vatech, Hwaseong, Republic of Korea) using the same parameters for all images: 0.2 mm voxel size; 12 × 8.5 cm field of view; 80 kVp, 3.7 mA; 14-bit depth; and 24 s acquisition time. For positioning, the equipment's laser alignment lights were used, with the median sagittal plane centralized and perpendicular to the ground. An expanded polystyrene block, 5 cm in height, was used as support for the skulls in the occipital condyles to

Figure 1 Axial reconstructions of the gold-standard skulls 1 (a), 2 (b) and 3 (c).

maintain the vertical angulation standardized between the acquisitions.

Skulls preparation

After the first image acquisition (Figure 1), the heads were fixed inside a container using metallic pins to do the molding and standardize the positioning. They were then molded using the Crislight resin (Siquiplás Ltd, São Paulo, Brazil) so that the space corresponding to the original soft tissues could be filled in with the simulant materials. The skulls had their soft tissues removed and replaced with the simulant materials, using the molds previously made. Three materials were tested: ice (Figure 2a); modelling wax (Figure 2b); and ballistic gelatin (Figure 2c). CBCT images for all the materials were acquired, following the same acquisition protocol as the gold standard (Figure 3).

Image evaluation and data analysis

The CBCT images were exported as DICOM (Digital Imaging and Communications in Medicine) to ImageJ v. 1.51m9 (U. S. National Institutes of Health, Bethesda, MD). Pixel intensity mean and standard deviation values were the obtained.

The image evaluation was done choosing regions of interest of 2 × 2 mm fixed square size in the axial reconstructions, using the other planes (Sagittal and Coronal) as reference, in the gold standard images (Figure 4). For

Figure 2 Skull with ice (A), with modelling wax (B), with the ballistic gelatin (C).

each head, 24 areas were selected: 12 in the maxilla and 12 in the mandible, involving anterior and posterior teeth including only dentin and pulp (Figure 4a), alveolar bone including only trabecular bone (Figure 4b) and soft tissues regions (Figure 4c), on the right and left sides. All regions were selected in standardized areas using anatomical landmarks as reference.

The simulants were compared to the gold-standard by the repeated-measures analysis of variance and Dunnett's test, considering each structure (tooth, bone, and soft tissue) separately, for both the mean pixel intensity and standard deviation values. The influence of the location (mandible or maxilla) was also analyzed by the same tests. The correlation between the values for the

gold standard and the different simulants was made by the Pearson's correlation test.

In addition, linear regression was used to establish the dependence between the gold standard mean pixel intensity values (or standard deviations) with the simulants. The significance level was set at 5% for all tests and the software used were BioEstat 5 and GraphPad Prism 7.

Results

The pixel intensity values of teeth regions were not affected by the simulant materials ($p = 0.1017$) in relation to the gold standard values (Figure 5A). For the alveolar bone (Figure 5B), the pixel intensity values obtained for gold standard values were higher than the ones obtained with ice ($p = 0.0156$), but they were not influenced by the modelling wax ($p = 0.8933$) and ballistic gelatin ($p = 0.4043$).

The mean pixel intensity values obtained in soft tissue regions for the gold-standard (Figure 5C) did not differ from the values obtained when ballistic gelatin was used as simulator material ($p = 0.5814$). However, both ice ($p = 0.0076$) and wax ($p = 0.0001$) presented lower mean pixel intensity values than the gold-standard.

The mean pixel intensities obtained with the simulants and gold standard were not influenced ($p > 0.05$) by the location (maxilla or mandible) for both tooth (Figure 6A) and bone regions (Figure 6B).

For the soft tissues regions, the modelling wax induced lower mean pixel intensity ($p = 0.0002$) than the gold-standard in the mandible, while ice ($p = 0.2469$) and ballistic gelatin ($p = 0.6366$) did not influence the values. Considering maxilla, only ballistic gelatin ($p = 0.9361$) did not present significant differences with the gold standard. Both ice ($p = 0.0165$) and modelling wax ($p = 0.0010$) caused a decrease in the mean pixel values (Figure 6C).

Correlation

There was no statistically significant correlation between the mean pixel values observed for gold standard and ice considering soft tissues, but modelling wax presented a

Figure 3 Axial reconstructions: gold-standard (a), ice (b), modelling wax (c), ballistic gelatin (d).

Figure 4 Axial reconstructions with ROI markers: anterior and posterior teeth (a), anterior and posterior alveolar bone (b), anterior and posterior soft tissues regions (c). ROI, region of interest.

moderate correlation and ballistic gelatin presented a good correlation, as shown in Table 1.

Linear regression

Table 2 shows that ice was not important for the regression model considering the teeth ($p = 0.1039$), bone ($p = 0.3165$) and soft tissues ($p = 0.7271$), as well as modelling wax, which was also not significant for the models of teeth ($p = 0.3415$), bone ($p = 0.1723$) and soft tissues

($p = 0.4545$). Only ballistic gelatin provided a significant model of prediction of the gold standard pixel intensities. Altogether, the results showed that ballistic gelatin was the best simulant in comparison with both wax and ice.

Standard deviation (SD)

There were no statistically significant differences between the simulants and the gold standard ($p = 0.1315$) for

Figure 5 Mean pixel intensities observed with the different simulants for tooth (A), bone (B) and soft tissues (C) structures. Centre bar, average; error bar, standard deviation. Distinct letters = statistically significant differences between the methods, considering each structure separately.

Figure 6 Mean pixel intensities obtained with the different simulants for tooth (A), bone (B) and soft tissues (C) according to the location (mandible or maxilla). Centre bar, average; error bar, standard deviation.

the SD values of the dental regions. However, the SD of pixel intensities for the bone were lower for the gold standard than for ice ($p = 0.0001$), modelling wax ($p = 0.0001$) and ballistic gelatin ($p = 0.0002$).

Considering the soft tissues, there were no statistically significant differences for SD values between the gold-standard and ballistic gelatin ($p = 0.7757$), and modelling wax ($p = 0.9487$) and ice ($p = 0.2180$). Table 3 shows standard deviation values.

Discussion

The X-ray beam attenuation, its direct influence on the pixel intensity values and the importance of simulating soft tissues in oral radiology research have been previously discussed in the literature. However, many studies did not use a gold standard to compare different simulant materials,^{6,7,9,11} a condition considered fundamental in a validation process.

Some studies have used reference standards, such as mean grey values of digital periapical radiographs from a database, which were later compared to the values

Table 1 Pearson's correlation between the gold standard and the simulants

Region	Ice	Modelling wax	Ballistic gelatin
Soft tissues	0.34 ($p = 0.1009$)	0.62 ($p = 0.0012$)	0.78 ($p < 0.0001$)

measured in two-dimensional images of anatomical pieces with different simulant materials.^{6,7} Other studies have aimed to evaluate the influence of materials on the grey values in the dental and bone regions when acquiring two-dimensional images of dry jaws and to compare these values between the groups.^{9,11}

Although one study compared the radiographic images of a skull prior to soft tissue removal and with different simulant materials,¹⁰ the thickness used for these materials was not the same as that of the original tissues. Therefore, these studies have some limitations, as they did not reproduce the original tissue characteristics, particularly the thickness.

The present study used piglet heads because their maxillomandibular structures are anatomically similar to those in human heads.¹² These fresh heads, with an

Table 2 Linear regression of mean pixel intensities for gold standard and simulants, for the tested regions

Regions	Variables	Coefficient	Standard error	Standardized p coefficient
Teeth	Constant	1806.8	254.5	—
	Gelatin	-0.79	0.28	-0.53
Bone	Constant	288.2	45.9	—
	Gelatin	0.55	0.06	0.87
Soft tissues	Constant	98.2	21.1	—
	Gelatin	0.58	0.09	0.80

Table 3 Standard deviation of pixel intensities of the different regions and materials

		<i>Simulant material</i>				<i>p</i>
		<i>Gold-standard</i>	<i>Ice</i>	<i>Modelling wax</i>	<i>Ballistic gelatin</i>	
Teeth	Mean ($\pm$ SD)	317.9 ($\pm$ 174.5)	395.1 ($\pm$ 216.1)	396.8 ($\pm$ 136.2)	385.1 ($\pm$ 172.8)	0.1315
	95% CI	242.5–393.4	301.7–488.6	337.9–455.7	310.4–459.9	
Bone	Mean ($\pm$ SD)	97.43 ($\pm$ 30.93)	173 ($\pm$ 81.92)	160.1 ($\pm$ 55.95)	140.4 ($\pm$ 45.63)	<0.0001
	95% CI	84.37–110.5	138.5–207.6	136.5–183.7	121.2–159.7	
Soft tissues	Mean ($\pm$ SD)	53.39 ($\pm$ 13.61)	61.79 ($\pm$ 23.81)	51.25 ($\pm$ 14.84)	49.55 ($\pm$ 13.94)	0.0706
	95% CI	47.64–59.14	51.74–71.85	44.99–57.52	43.66–55.43	

CI, confidence interval; SD, standard deviation.

excellent state of preservation (no hard or soft tissue damage), were considered as the gold standard for the present study. The same thickness of the original soft tissues could be reproduced with the simulant materials tested, suggesting that a real condition might have been more closely represented.

Different from previous studies, which used two-dimensional radiographs,^{6–8,10,11} the present study was aimed at CBCT images, which is a three-dimensional image modality that results in high quality images and thus contributes significantly to the diagnosis. Also, it has no geometrical limitations inherent in two-dimensional techniques.¹³

Pixel intensities were measured using the ImageJ software, which is used in many studies on oral radiology and provides free access, reproducibility, and histogram evaluation tools.^{11,14–16} This made it possible to compare the results with other studies that had similar methodology.

Despite the limitations towards voxel values, many studies have evaluated the influence of field of view size, exposure parameters and dental implants on such values.^{3,14,16,17} To evaluate their variability, some authors measured several regions of interest in the image to obtain the mean and standard deviation among these regions.^{2,3,18}

The literature makes it clear that voxel values vary according to the location in the scanned volume.^{11,19} Therefore, in the present study, measurements were made in specified regions of the maxillomandibular complex, a condition different from those reported in previous studies which did not specify the regions analyzed.^{6,10} Thus, it was possible to evaluate the influence of the simulant materials on pixel intensities among the teeth, bones, soft tissues and their location (maxilla and mandible). This, in fact, was observed in this study, as the values of these regions presented variations among themselves.

CBCT equipment uses specific greyscale intensities, which can generate numerically distinct values when comparing different CBCT volumes. In the present study, image acquisitions were done in the same equipment (14-bit depth). The need for normalizing the values for comparison was discarded since the images compared had the same contrast resolution. However,

to compare values of different equipment, a linear correlation between the values should be performed.^{1,2}

The soft tissue simulant materials most commonly reported are wax^{6,7,10,11} and liquid water,^{6,8,11} due to their low cost, ease of handling, and great accessibility,⁷ factors that accounted for their use in the present study. Ballistic gelatin has also been widely tested as a human tissue simulant, particularly in forensic research. The choice of ballistic gelatin was based on its physical characteristics that simulate the density and viscosity of muscle tissues.^{20,21} No other study was found to evaluate its influence on radiographic intensity values.

The present study used frozen water to comply with the recommended methodology. The modelling wax was chosen instead of utility wax due to its ability to simulate gingival tissues in prosthetic dentistry.²²

Studies have shown satisfactory results for wax as a simulant material,^{6,10,11} a finding in accord with that of the present study, detecting its influence on the pixel intensities for the dental and bone regions. Schropp *et al*⁷ reported wax as a good soft tissue simulant by comparing the radiographic density of 61 human cheeks with the images of a wax block, whose thickness was not the same as that of the soft tissues used as reference. However, in the present study, when evaluating the soft tissue region, the wax did not present itself as a good simulant when compared to the gold standard.

Disagreement between studies can be explained by methodological differences. In the present study, the same thickness of the original soft tissues was used, showing the need for the simulant material to have similar density to that of the original soft tissues, so that they behave in a similar way. Besides the difference in methodology, it is also hypothesized that the CBCT may have generated artefacts in the image and consequently altered the values.

Water has been considered a good soft tissue simulant, when its influence on grey values concerning teeth and bones was analyzed.^{6,8,11} This was also observed in the present study for the tooth region, but not bone and soft tissue regions. In the bone region, because it was in direct contact with the head, water might have infiltrated the medullary spaces, consequently altering the grey values. Further studies are needed to investigate the influence of water density on image quality, especially

those involving soft tissues. However, it is believed that frozen water, which has a lower density (0.93 g cm^{-3}) when compared to liquid water (1.0 g cm^{-3}),²³ has less interaction with radiation and, consequently, results in fewer artefacts.²⁴ In the present study, despite its thickness being the same as that of the original soft tissues, the ice showed no satisfactory results, a finding that can be justified by the different densities of the ice and the original soft tissues.

Farquharson *et al*⁸ reported hydrophilic materials as good soft tissue simulants, a finding in accord with that observed in our study, where the ballistic gelatin showed good results in all the regions tested. This can be explained by the presence of collagen in its composition, contributing to the similarity between the material and soft tissues densities.

Results of the present study showed differences in how the simulant materials behaved in different regions. However, the purpose of each assessment was different. In the dental and bone regions, the simulant materials were evaluated for their influence on the intensity values as an attenuator.¹¹ Analysis of the mean pixel intensity values showed that the simulant materials tested had no statistically significant influence on such values concerning the tooth region. In the bone region, however, water presented different values.

The simulant materials for the soft tissue regions were evaluated for their ability to reproduce the intensity values of the original tissues, not just as an attenuator. In fact, among the simulants tested, only the ballistic gelatin showed good results, probably because its density is most similar to that of the original tissues. However, artefacts were clearly visualized in the images,

as the gelatin density is lower than that of the tooth and bone regions. Further studies are needed to measure the intensity in the soft tissues; this was the first study to evaluate such variable in CBCT images.

The differences among the simulants were more evident for the soft tissues when the influence of the location was assessed. This may be related to the differences in soft tissue thickness of the anterior and posterior regions of the mandible and the maxilla.

Although all the simulant materials tested did not statistically influence the values of the dental region, and only ice compromised the values in the bone region, ballistic gelatin was the only material that presented good results overall. The logistic regression showed, in fact, that only ballistic gelatin was able to adequately reproduce the pixel intensities of the gold standard. The analysis of the standard deviations also showed similar variability values for the gold standard and all the simulants.

Based on our results, among the three tested materials, the ballistic gelatin was the best soft tissue simulant. Although ice and wax had no statistically significant influence on the pixel intensity concerning the tooth and bone regions, these materials were not considered good soft tissue simulants.

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