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RESEARCH

Comparison of changes in dental and bone radiographic densities in the presence of different soft-tissue simulators using pixel intensity and digital subtraction analyses

R S de Molon*[,1], R G Batitucci[1], R Spin-Neto[2], G M Paquier[1], C E Sakakura[3], G M Tosoni[1] and G Scaf[1]

[1]Department of Diagnosis and Surgery, School of Dentistry at Araraquara, Univ Estadual Paulista, Araraquara, São Paulo, Brazil; [2]Oral Radiology, Department of Dentistry, Aarhus University, Aarhus, Denmark; [3]Department of Implantology, Barretos Dental School, Barretos Educational Foundation, São Paulo, Brazil

Objectives: To evaluate the influence of soft-tissue simulation materials on dental and bone tissue radiographic densities using pixel intensity (PI) and digital subtraction radiography (DSR) analyses.
Methods: 15 dry human mandibles were divided into halves. Each half was radiographed using a charge-coupled device sensor without a soft-tissue simulation material (Wm) and with 5 types of materials: acrylic (Ac), wax (Wx), water (Wt), wood (Wd) and frozen bovine tissue (Bt). Three thicknesses were tested for each material: 10 mm, 15 mm and 20 mm. The material was positioned in front of the mandible and the sensor parallel to the molar region. The radiation beam was perpendicular to the sensor at 30 cm focal spot-to-object distance. The digital images of the bone and dental tissue were captured for PI analyses. The subtracted images were marked with 14 landmark magnifications, and 2 areas of analyses were defined, forming the regions of interest. Shapiro–Wilk and Kruskal–Wallis tests followed by Dunn's post-test were used ($p < 0.05$).
Results: DSR showed that both the material type and the thickness tested influenced the gain of density in bone tissue ($p < 0.05$). PI analyses of the bone region did not show these differences, except for the lower density observed in the image without soft-tissue simulation material. In the dental region, both DSR and PI showed that soft-tissue simulators did not influence the density in these regions.
Conclusions: This study showed that the materials evaluated and their thicknesses significantly influenced the density-level gain in alveolar bone. In dental tissues, there was no density-level gain with any soft-tissue material tested.
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Introduction

In vitro radiographic studies provide valuable and accurate diagnostic information in dental research.[1] In those studies, soft-tissue simulators are often used to simulate what is present *in vivo*.[2] In a clinical situation, when an X-ray beam interacts with soft tissues and reaches the object, its intensity is attenuated, resulting in low-energy photons producing low-density areas in the image.[3] The use of soft-tissue simulation materials having densities similar to the tissues found in patients in a real environment helps avoid exposure

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*Correspondence to: Dr Rafael Scaf de Molon, Department of Diagnosis and Surgery, School of Dentistry at Araraquara, Universidade Estadual Paulista, Rua Humaitá, 1680, 14801-903 Araraquara, São Paulo, Brazil. E-mail: molon.foar@yahoo.com.br
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of patients to excessive radiation during *in vivo* studies.[4]

Based on literature, the use of soft-tissue simulators, such as acrylic plates,[2,4–8] wax,[2,4,9,10] water,[11–14] wood[4,15,16] and bovine tissue[17] has been tested in different ways with respect to the nature and/or the thickness of the material put around dry mandibles.[2,5,16] It is important that these simulation materials are accurately reproducible, measurable and are ready for use in any circumstances.[18] The influence of soft-tissue simulators on mandible bone radiographic density has been shown previously using digitial intraoral radiography.[17] In a recent article, Schropp et al[2] evaluated the thickness of wax and acrylic used as soft-tissue simulation materials to obtain a radiographic density similar to that of the human cheek. This study showed that acrylic and wax with thicknesses of 14.5 mm and 13–17 mm, respectively, could simulate the human cheek in *in vitro* radiographic studies.

Digital imaging helps analyse bone density and architecture quantitatively and qualitatively[19] and has advantages over film-based radiography, which is mainly connected to smaller radiation doses.[20] Digital images are composed of an array of pixels. Pixel intensity (PI) analysis is a measure of the blackness and whiteness on a scale from 0 (totally black) to 4096 (totally white)[19] and could be a useful and simple method to evaluate and measure the bone density in mandibles and maxillae.

Also, digital imaging makes the analyses of dental and bone density using digital subtraction radiograph (DSR) possible. In this context, DSR detects subtle changes in alveolar bone in the radiographic image, being a highly sensitive methodology,[21] improving the detection of periodontal lesions. DSR is also used for the evaluation of teeth with carious lesions[22] and dental implants.[23] This method consists of a subtraction operation between two sequential radiographs in which structures that do not change are eliminated. Thus, DSR increases the ability to detect small changes in the radiographic image, improving the detection of bone alterations and reducing the interference of anatomical structures.[24,25] However, digital subtraction method is still not routinely used in clinics because of standardization problems, and most of the available literature is in respect to *in vitro* and *ex vivo* studies.

To the best of our knowledge, there are no studies that correlate different soft-tissue simulation materials and thicknesses for radiographic dental and bone densities using PI and DSR. Therefore, the purpose of this study was to evaluate the influence of five soft-tissue simulation materials of three thicknesses on dental and bone tissue radiographic densities using PI and DSR, with an aim to facilitate radiological experiments.

Material and methods

The sample of this study comprised 15 partially edentulous dry human mandibles from the Department of Morphology, Laboratory of Anatomy, Araraquara Dental School, Araraquara, Brazil. The mandibles were divided into halves; thus, the selected sample size was 30 dry hemimandibles. Inclusion criterion was the presence of at least two teeth in the posterior mandibular area.

Soft-tissue simulator materials

For the soft-tissue simulations, 5 types of materials were tested: acrylic (Ac), wax (Wx), water (Wt), wood (Wd), and bovine tissue (Bt), of 3 thicknesses: 10 mm, 15 mm and 20 mm, and in a standard size of 16 × 16 cm.

For the simulation using water (Wt), three acrylic containers were made with the same dimensions of height and width but with varying depths, according to the thicknesses tested, and were filled with water. The walls of the acrylic containers were made as thin as possible (2 mm) to avoid interference with the results. Samples of bovine tissue (Bt), muscle and fat were packed in a thin plastic wrapper and frozen and made to the same dimensions in height and width.

Image acquisition

The posterior region of each hemimandible was radiographed using a dental unit (Oralix 765 DC; Gendex, Milan, Italy) and a charge-coupled device (CCD) sensor system (Visualix® eHD; Gendex, Milan, Italy) without material (Wm) and with the five tested materials, in the three tested thicknesses. The hemimandibles were fixed and stabilized using an appropriate device, and the material was positioned in front of the mandible. The sensor was placed parallel to the molar and/or premolar regions. The X-ray beam was positioned perpendicular to the mandible, sensor and soft-tissue simulators, at a 30 cm focal-spot-to-sensor distance, to standardize image acquisition. The exposure settings were 65 kVp, 7 mA and 0.08 s (Figure 1). The digital images were captured and saved at a resolution of 1300 dpi and depth of 8 bits and 16 bits and stored in the tagged image file format. Neither the sensor nor the mandible

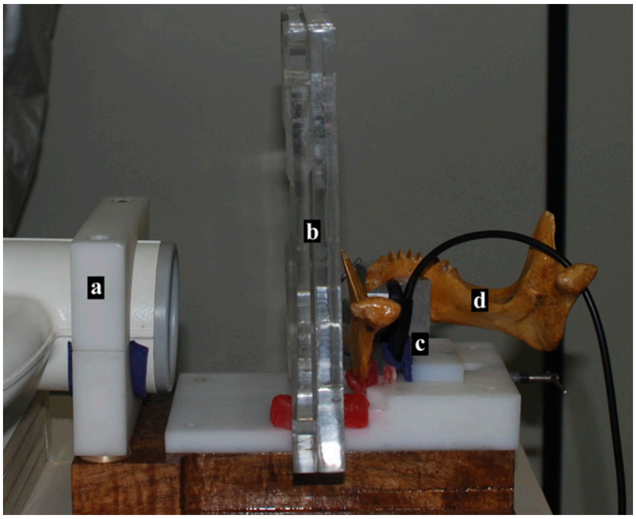


Figure 1 Exposition standardization: (a) X-ray cylinder, (b) soft-tissue acrylic material, (c) digital sensor and (d) mandible

was moved during the experiment (only the soft-tissue simulation material was changed between recordings).

Pixel intensity analyses

The images obtained with depths of 8 bits and 16 bits were analysed using a dedicated software (ImageJ; National Institutes of Health, Bethesda, MD) for calculating the PI in 2 independent regions—bone and dental tissue. The area was limited to a square of 100 × 100 pixels in the radiographic image. For the evaluation of bone tissue, the region of interest (ROI) was delimited between the tooth roots and called Region 1 (Figure 2a). For the evaluation of dental tissue, the ROI was delimited between the healthy dental crown comprising the enamel and dentin and called Region 2 (Figure 2b).

The coordinates of each ROI were recorded, generating a macro for each jaw and each region, which was repeated for all images of the same hemimandible, to ensure that the same ROI was measured in all images. The average grey levels of the corresponding ROIs were analysed for both 8 and 16 bit images in the two ROIs, to compare the influence of the tested soft-tissue simulators on bone and tooth structure radiographic densities and if the depth of the materials would influence the pixel intensity results.

Digital subtraction analyses

For DSR analyses, the images were exported to a digital subtraction software (X-Poseit®, v. 3.1.17; Image Interpreter System, Lystrup, Denmark). 2 digital images, 1 Wm and the other with 1 simulator material (Ac, Wx, Wt, Wd or Bt, in 3 diverse thicknesses—10 mm, 15 mm and 20 mm) were imported to the software and positioned side by side (Figure 3). Thus, the initial image was always the image without material (Wm), as the final image was the image with a soft-tissue simulator material, generating a subtracted image (Figure 4). To prepare the baseline (Wm) and the final image for the subsequent subtraction, 14 reference points were positioned in diverse places at the border of cement–enamel junction, enamel, dentin, and alveolar bone crest. The precision of the reference points placed in the two radiographs was evaluated by means of an accuracy tool in the software. The points were repositioned if the distance between them in the two images was larger than 2 pixels.

To restrict the areas of analyses to those interesting for the study, the following ROIs were outlined using the computer mouse:

Region of interest: R1 related to the alveolar bone around the dental root and R2 related to the dental tissues. 1 rectangular window with 99 × 225 pixels and 1 square window with 73 × 73 pixels, respectively, were drawn for R1 and R2.

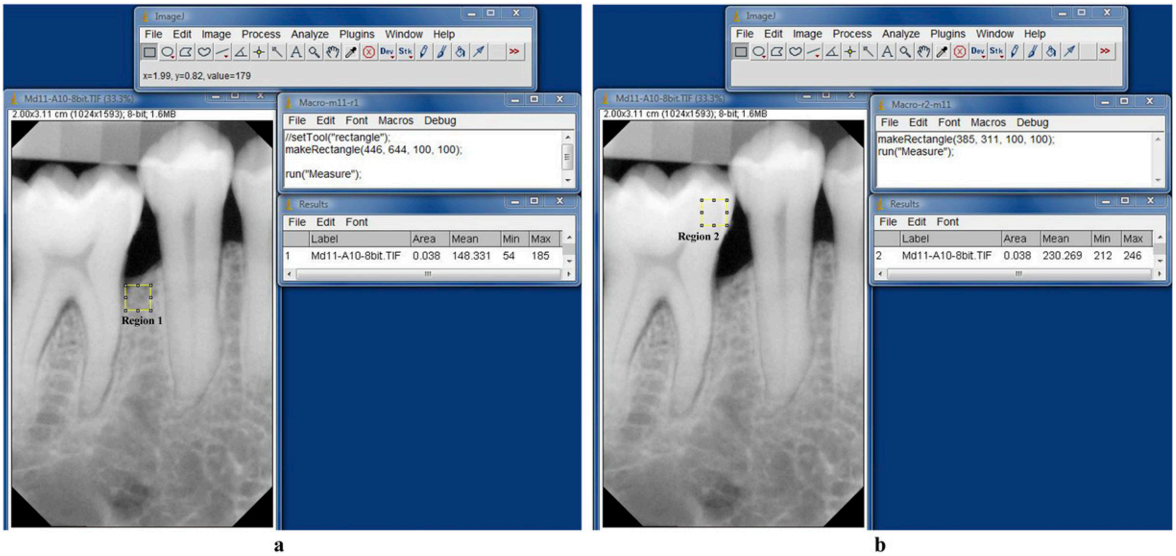
Region of control: Two rectangular areas were drawn including the two areas of interest: dental tissue and alveolar bone, to take into account the noise in the image, *i.e.*, recording errors not accounted for by the program.

ROI and region of control selected were made on the baseline radiographs and automatically transferred to the subtraction images.

The standard deviation of the histogram distribution for the shades of grey in the region of control of the subtraction image was used to determine the threshold between what was interpreted as a signal and what could be determined as a noise in the subtraction image.[26] The software performed the subtraction automatically, and all data concerning the subtraction in ROIs were automatically stored in the program data bank.

Data analyses

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).



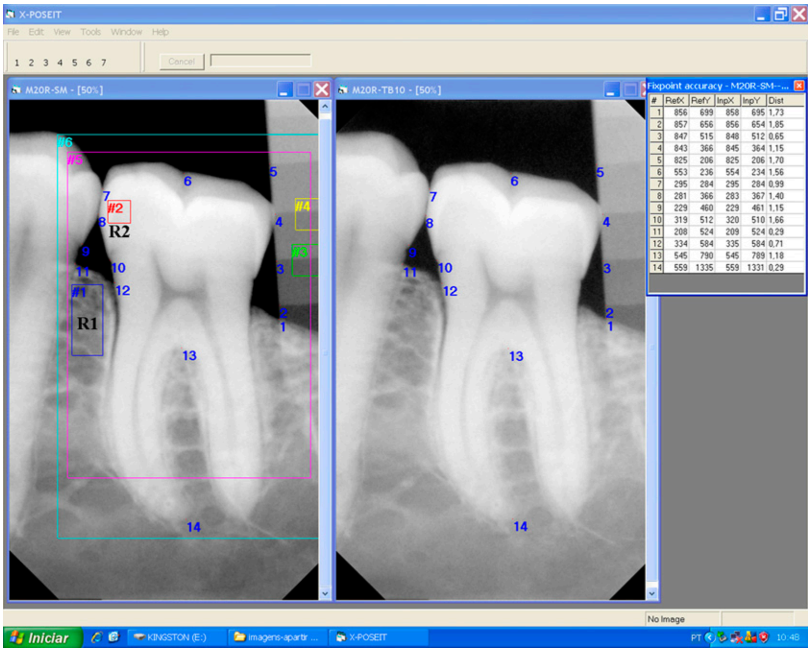
```
//setTool("rectangle");
makeRectangle(446, 644, 100, 100);

run("Measure");
```

```
    Label               Area    Mean      Min   Max
1   Md11-A10-8bit.TIF   0.038   148.331   54    185
```

```
makeRectangle(385, 311, 100, 100);
run("Measure");
```

```
    Label               Area    Mean      Min   Max
2   Md11-A10-8bit.TIF   0.038   230.269   212   246
```

Figure 2 Pixel intensity analysis in the ImageJ program (National Institutes of Health, Bethesda, MD) showing (a) Region 1, in bone tissue, and (b) Region 2, in dental tissues



Fixpoint accuracy - M20R-SM--...

#	RefX	RefY	InpX	InpY	Dist
1	856	699	858	695	1,73
2	857	656	856	654	1,85
3	847	515	848	512	0,65
4	843	366	845	364	1,15
5	825	206	825	206	1,70
6	553	236	554	234	1,56
7	295	284	295	284	0,99
8	281	366	283	367	1,40
9	229	460	229	461	1,15
10	319	512	320	510	1,66
11	208	524	209	524	0,29
12	334	584	335	584	0,71
13	545	790	545	789	1,18
14	559	1335	559	1331	0,29

Figure 3 Examples of the reference points marked using X-poseit, v. 3.1.17 (Image Interpreter System, Lystrup, Denmark) subtraction program. 14 reference points were positioned at the border of cement–enamel junction, enamel, dentin, and alveolar bone crest, standardizing the subtracted images. R1 is representative of the alveolar bone around the dental root and R2 related to the dental tissues

The results were comparatively evaluated in the different groups for all analysed parameters. Shapiro–Wilk and Kruskal–Wallis tests followed by the Dunn's multiple comparisons post test were used to determine any significant differences between the groups, at a 5% significance level.

Results

Pixel intensity

The results showed that no statistical difference was observed for PI among the diverse materials and thicknesses that were tested, in both Region 1 (with 8 and 16 bit images, respectively; Figure 5a,b) and Region 2 (with 8 and 16 bit images, respectively; Figure 6a,b). However, there was a statistically significant difference between the PIs obtained with the simulation materials and the absence of a simulator material in Region 1 (bone tissue, for both 8 and 16 bit images). In Region 2 (dental tissue), there was no significant difference in the presence or the absence of a soft-tissue simulator. This result demonstrates that the material used interfered in the X-rays attenuation, increasing the PI in the bone region.

Digital subtraction radiography

DSR analysis for bone tissue showed that for a thickness of 10 mm, Ac, Wt and Bt presented a significantly higher gain of grey levels than Wd and Wx. For a thickness of 15 mm, the tested material did not influence grey-level gain for Ac, Wt and Wd, whereas the Bt group presented a higher grey-level gain than the Wx group. For the thickness of 20 mm, Bt showed significantly higher grey-level gain, whereas Ac and Wd presented lesser grey level gain, compared with all other tested materials (Figure 7a). In general, Bt presented higher grey-level gain in the 3 tested thicknesses (Figure 8).

Density gain area (by means of DSR) in R1 showed that the different soft-tissue simulator materials and thicknesses tested did not influence what was assessed for bone. Therefore, there was no statistically significant difference between the materials and thicknesses tested in this region (Figure 7b). In R2, there was not enough gain of grey-level data to perform any statistical analysis because we found a majority of null results.

Discussion

In this study, we used PI and DSR to evaluate the influence of soft-tissue simulation materials in the determination of dental and bone densities. To simulate soft tissues, we used five types of materials in three diverse thicknesses. Both material type and thickness have been widely used[2,5,6,9,10,15,16] with aims to simulate the real anatomical structures.[27,28] In this study, we showed that both the material type and the thickness tested influenced the gain of density in bone tissue as demonstrated by DSR. PI analysis in the bone region showed low density in the image without simulating material. In the dental region, both DSR and PI revealed that soft-tissue simulators did not influence the teeth density.

DSR is often used to detect subtle changes in bone and dental structures. However, the reproducibility of geometric factors on the images to be subtracted is

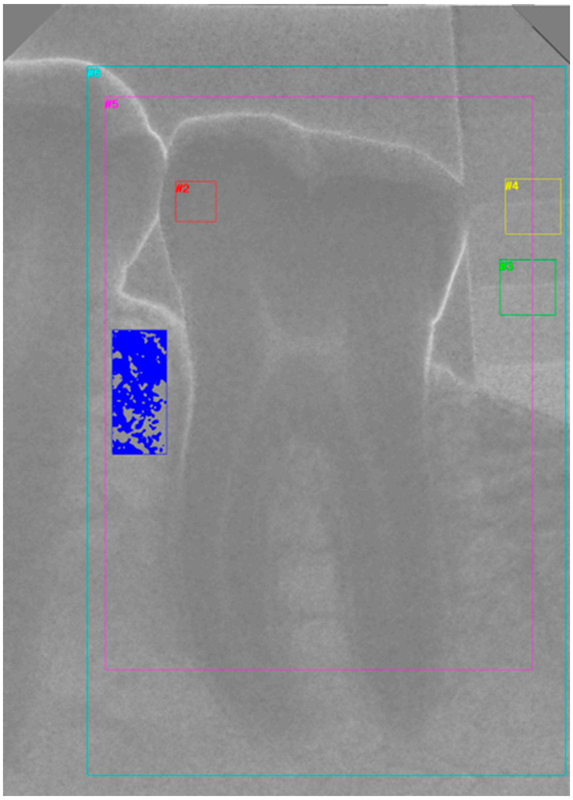


Figure 4 Representative subtracted image

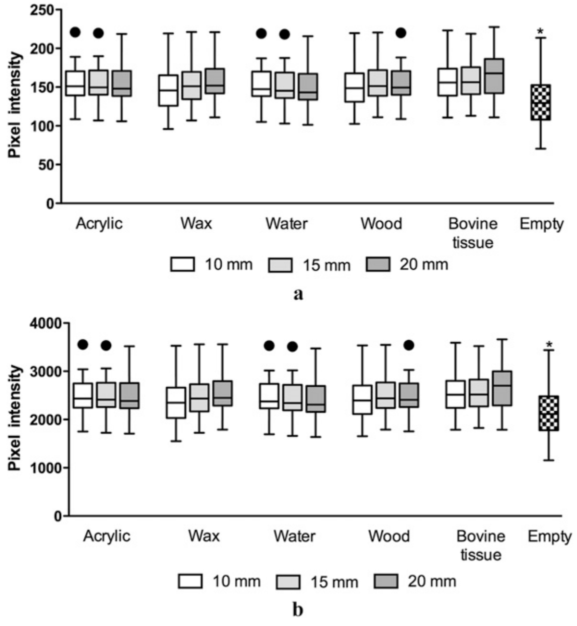


Figure 5 Pixel intensity gain of grey among different soft-tissue materials of each thickness in Region 1 with (a) 8 bits and (b) 16 bits (Kruskal–Wallis test–Dunn's test). * indicates significant statistical difference ($p < 0.05$). • represents the outliers—data more than two standard deviations

essential for accurate digital subtraction radiography.[29,30] Therefore, the digital sensor system (CCD) was chosen for being a direct digital radiographic system that does not need to be removed after exposure to an X-ray beam, assuring the standardization of geometric factors during the experiment.

When an X-ray beam passes through soft tissues, some of these photons are absorbed by the tissues and some have its trajectory altered, leading to a dispersion or scattering of the beam, changing the incidence in radiographic film or sensor and, therefore, the measured radiographic density. The phenomenon of attenuation depends on atomic interactions, so materials with diverse compositions may lead to different degrees of attenuation, described as linear attenuation coefficient.[3] As radiographic tissue density is derived from this attenuation coefficient degree, quantification of the density becomes more accurate when the influence of soft-tissue simulator materials is taken into consideration.[17]

According to Souza et al,[17] different materials with the same thickness showed different optical densities in the mandibular bone area, using digitized film-based radiographic images. However, the differences with the methodology used in our study make it difficult to compare the findings. PI results for Region 1 showed that soft-tissue materials and thickness did not affect the radiographic density of the bone tissue. On the other hand, when there was no soft-tissue simulator material, the pixel intensity was lower, influencing the grey-level values. Our results suggest that PI analysis of a specific ROI is a simple and efficient method that may be applicable to an analysis of density level in digital images.

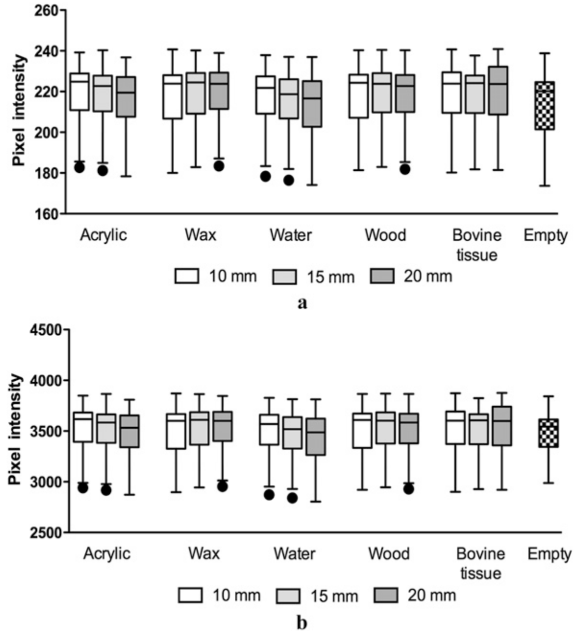


Figure 6 Pixel intensity gain of grey among different soft-tissue materials of each thicknesses in Region 2 with (a) 8 bits and (b) 16 bits (Kruskal–Wallis test–Dunn's test). • represents the outliers—data more than two standard deviations

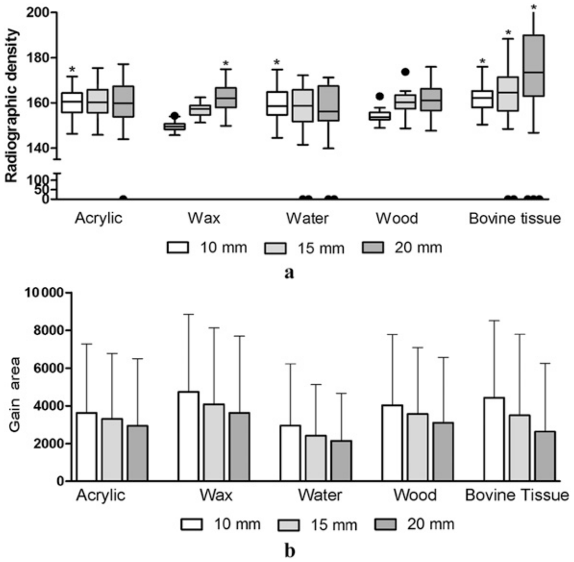


Figure 7 (a) Digital subtraction radiography analyses among different soft-tissue materials of each thickness in Region 1 (Kruskal–Wallis test–Dunn's test). * indicates significant statistical difference compared with the others ($p < 0.05$). (b) Digital subtraction analysis of gain area among the different soft-tissue simulator materials of three thicknesses. • represents the outliers—data more than two standard deviations

In Region 2 (for PI) and R2 (for DSR), our results showed that the materials and thicknesses tested presented no statistically significant difference in density gain. This must be considered carefully when dealing with cavitation or demineralization detection. In studies related to dental caries,[6,10,28,31] the simulation of soft tissue can lead to a reduction in radiographic image contrast, impairing the diagnosis. In this way, we cannot extrapolate the present results to that type of clinical situation.

This finding may be related to the high density (lighter images) observed in the dental tissue radiographs, mainly for enamel. However, the attenuation of X-ray beams for soft-tissue simulation materials may interfere with the image of low-density structures, such as a bone. The bone marrow spaces in the radiographic image presents lower radiographic density. Thus, the attenuation of X-ray beams in the regions of bone, promoted by the interposition of soft-tissue simulation materials, is more noticeable than those of enamel and dentin. If the study includes teeth with cavitation or demineralization process, the loss of dental material might set the experiment more susceptible to density changes caused by the soft-tissue simulators.

DSR could detect different grey-level gains among the soft-tissue simulator materials and thicknesses. Our results showed that bovine tissue as a soft-tissue simulator material presented the highest grey-level gain compared with the others. These results are in accordance with a previous study.[17] Furthermore, Wx, Wd and Bt showed an increased grey-level gain according to the increase in thickness.

In this experimental model, there was no difference between the materials and thicknesses related to the gain area analysed with DSR. A possible explanation for these results would be the exposure latitude of the used CCD sensor. A previous article[32] has shown a wide range of latitudes among 18 X-ray detectors, including the 1 used in the present study and film radiographs. Moreover, photostimulable storage phosphor plate systems were shown to possess even wider latitudes than solid-state sensors. This variation of the dynamic range between film and digital systems may give rise to the assumption that a different result would have been obtained if a film was used in the present study because subtle differences in pixel intensity/image density related to the various types of simulation materials may have been revealed with a film. However, the use of digital imaging is increasing and replacing film-based radiography, and it is likely that most future assessments of new radiographic detectors using an *in vitro* model will be on digital receptors.

Another aspect to be considered is that despite the assumption that conventional radiographic film would be able to present statistically significant differences between the use/non-use of soft-tissue simulation materials, the use of direct digital radiographic system has increased in recent years, and therefore it is necessary to know the differences that occur among the diverse radiographic digital systems. In this way, it is important to emphasize that the results of the present study should be considered specific to the intraoral digital system evaluated.

In conclusion, according to the limits of this study, the materials tested were showed to significantly influence grey-level gain in the alveolar bone, especially when using Bt. The thickness of the soft-tissue simulator tested also influenced the gain of radiographic density, in alveolar bone, except for Ac and Wt. In dental tissues,

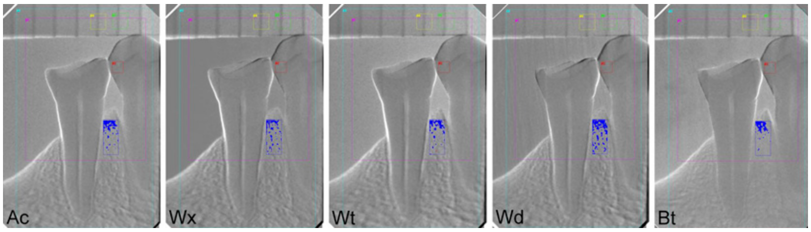


Figure 8 Representative subtracted images of 5 soft-tissue materials with thickness of 10 mm: acrylic (Ac), wax (Wx), water (Wt), wood (Wd) and bovine tissue (Bt)

there was no grey-level gain with any soft-tissue simulator material tested, suggesting that it is not necessary for any type of material to simulate the soft tissues in teeth, and further studies, considering teeth with cavitation or demineralization processes, should be carried out.

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