Microcomputed Tomography Calibration Using Polymers and Minerals for Enamel Mineral Content Quantitation

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Significance of the Study

- This study developed calibration standards (CSs) that are readily available for clinical researchers for the quantitation of enamel mineral content. It employed polymers and minerals as CSs in lieu of expensive and difficult-to-fabricate traditional standards. This simple method could encourage clinicians to use this technology to expand knowledge in the field of caries research.

Keywords

Microcomputed tomography · Calibration standards · Minerals · Polymers · Early enamel lesions

Abstract

Objective: The aim of this paper was to develop calibration standards (CSs) that are readily available for clinical researchers for the quantitation of enamel mineral content. **Method:** Polyethylene terephthalate (PET), acetal, polyphenylene sulfide (PPS), selenite, Egyptian alabaster, aragonite, and fluorite were fashioned into discs, and their densities were measured and stacked for microcomputed tomography examination. Frame averaging, flat-field correction, pre-filtration, and beam-hardening correction were applied. CSs were checked for homogeneity. The linear relationship between the mean greyscale value (GSV) of each disc and its physi-

cally calculated density was explored, and reproducibility was tested. A calibration function was established and then validated using a bovine enamel disc and sound enamel of extracted human premolar teeth. **Results:** Measured densities were PET ($\rho = 1.38 \text{ g/cm}^3$), acetal ($\rho = 1.41 \text{ g/cm}^3$), PPS ($\rho = 1.64 \text{ g/cm}^3$), selenite ($\rho = 2.24 \text{ g/cm}^3$), Egyptian alabaster ($\rho = 2.7 \text{ g/cm}^3$), aragonite ($\rho = 2.72 \text{ g/cm}^3$), and fluorite ($\rho = 3.11 \text{ g/cm}^3$). Examination of the profile sections of CSs confirmed the uniformity of GSVs with minimal beam-hardening effect. A squared Pearson correlation coefficient of $R^2 = 0.994$ was determined between the mean GSV of each CS and its calculated density and was reproduced at different settings with $R^2 > 0.99$. A linear regression equation of density (y) versus GSV (x) was established using the least squares regression equation method. The estimated density of the bovine enamel disc (2.48 g/cm^3) showed high accuracy when compared to the physically measured value (2.45 g/cm^3). The

relative error was 1.2%. Densities of sound enamel in the extracted human premolar teeth were 2.6–3.1 g/cm³. **Conclusions:** This is a simple, valid, and reproducible method to quantitate enamel mineral content. This simple, yet accurate system could be used to expand knowledge in the field of enamel caries research.

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Introduction

Microcomputed tomography (micro-CT) technology, and more recently, nanocomputed tomography technology, enable three-dimensional, nondestructive imaging of tooth structures revealing microscopic details [1–4]. This approach has been used to study the demineralization and remineralization of teeth [5–11]. It can also be used to study before and after comparisons of different dental treatments [12–14]. However, as other methodologies, micro-CT imaging has its limitations and challenges [15]. A potentially useful application of micro-CT imaging is the determination of mineral density based on reconstructed greyscale values (GSVs) of micro-CT image voxels. While the GSVs of structures visible in micro-CT scans have been shown to be proportional to the mineral density of scanned materials [16–18], the calibration of these values is a prerequisite for valid quantitation. With the possibility of machine X-ray source and detector sensitivity drift and setting variations between scans, researchers need a method for external calibration between and within their scans [19].

The focus of this study was to develop calibration standards (CSs) that are handy for clinical researchers to use for quantitating enamel mineral content. Hence, this communication covers only enamel calibration, and it summarizes some of the challenges encountered with the existing calibration methods. Metals, such as aluminum, have been traditionally used to calibrate microradiography and micro-CT [7, 20, 21]. An aluminum step wedge has been used to calibrate beam attenuation to enable accurate mineral content calculations. The drawback of this technique is the fact that commercial desktop micro-CT systems utilize polychromatic radiation where the beam has altered X-ray energies, leading to different attenuations of the same material [16]. Another popular way to calibrate GSVs is the use of CSs, which are scanned together with the specimens. Pure pressed and sintered hydroxyapatite standards in solid form were proven to be spatially homogeneous [22, 23]. However, they are difficult to fabricate [23]. Other CSs made from hydroxyapa-

tite/resin mixtures typically cover the density range useful for studying bone, dentine, and other tissues with low mineral density but may not be suitable for tooth enamel research as higher densities are difficult to achieve and are often nonhomogeneous and fragile [19].

Our intention in this study was to find easy-to-fabricate materials for clinical researchers to use as CSs for research on enamel caries. These CS materials should have densities within the range of 1.52–3.14 g/cm³.

Materials and Methods

Seven solid materials were tested: polyethylene terephthalate (PET), acetal, polyphenylene sulfide (PPS), selenite, Egyptian alabaster, aragonite, and fluorite. They were fashioned into discs of a 9-mm diameter and 1.5-mm height to fit into a 2.0-mL vial (Nalgene® cryogenic vials). A digital caliper (accurate to 0.0001 mm; Mitutoyo Corporation, Aurora, IL, USA) was used to measure the dimensions of each disc to calculate the disc volume using the cylinder volume formula ($V = \pi r^2 h$). Five readings from different points on the discs were averaged to calculate the mean diameter and height. Mass was determined using an electronic, analytic scale (accurate to 0.0001 g; Shimadzu Corporation, Tokyo, Japan). The density of each disc was calculated using the formula ($\rho = m / V$, where m = mass and V = volume).

The CSs were stacked in a safe-lock, 2-mL vial and then stabilized with sticky wax on the rotation mount of the micro-CT system (Phoenix nanotom®m; GE, Germany) that rotates around an axis perpendicular to the beam direction (Fig. 1a). X-rays were generated at 110 kV and 160 μ A, creating 2,000 two-dimensional projections over a 360-degree rotation of the specimen with a voxel size of 13.3 μ m. A 0.25-mm-thick copper filter was placed in the path of the beam to restrict spectral bandwidth of the polychromatic radiation from the tungsten anode. Three-frame averaging was applied. Acquired images were 3,052 $\times$ 2,400 pixels in resolution. On average, the acquisition time was 105 min. The created 2D images were 3D reconstructed using Phoenix Datasx CT Software (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) and transferred to VGStudio Max 3.0 (Volume Graphics, Heidelberg, Germany) for visualization, segmentation, and analysis. The beam-hardening correction module of the Phoenix Datasx CT Software was applied during reconstruction (set at 8). These settings were optimized based on pilot experiments. The Phoenix nanotom®m machine also did an automatic beam-hardening correction, which aided in minimizing this artifact. To verify the homogeneity and uniformity of the CSs, a centered, cross-sectional 2D image was taken from the generated data set, across each CS. A Profile window was created, and a line was drawn across the CS's diameter creating a graph of distance (x) versus GSV (y). The created graph was also used to check for presence of beam hardening. Standard deviations were used to reflect the amount of noise present.

A representative volume chosen from the center of each CS was selected using ellipse selection mode and was dragged into the third dimension. A region of interest (ROI) was then created. Under the Porosity/Inclusion Analysis module, a defect analysis was performed for each ROI representing CS to obtain the mean and the standard deviation of GSV of each CS. The linear relationship be-

Fig. 1. CSs setup for micro-CT examination. **a** The seven tested CSs (from bottom to top): A: PET; B: acetal; C: PPS; D: selenite; E: Egyptian alabaster; F: aragonite; and G: fluorite. **b** The three chosen CSs (A: PET; D: selenite; and G: fluorite) scanned with an extracted human tooth.

tween the mean GSV of each disc and its calculated density was thereafter explored using the squared Pearson correlation coefficient (R^2). The reproducibility of the obtained linear relationship at different energy levels was tested through fixing the current at 160 μ A and varying the voltage from 60 to 110 kV. A scatter plot was created by plotting the mean GSV of each CS (x) against its' calculated density (y), and a calibration function was established using the least squares regression equation method ($y = \text{slope} \times x + \text{intercept}$).

Validation of the calibration function was done by estimating the density of a bovine enamel disc based on its average CT-determined GSV and comparing it to the physically measured value. The bovine enamel disc was obtained from the labial surface of an extracted bovine central incisor. A cylindrical piece of enamel with underlying dentin was cut using an 8-mm-diameter diamond core drill bit. Dentin was then removed, and the disc was flattened by wet sanding on 320 grit wet/dry sand paper. The bovine enamel disc was scanned on top of the CSs under the same experimental conditions that the calibration function was developed. The relative error for the CT-determined density was calculated.

Validation of the calibration function by estimating the mineral density of sound human enamel was done on 10 unrestored, non-cavitated, extracted human premolars with closed apices. Teeth with developmental enamel defects were excluded. Extracted teeth were collected from several dental clinics and were stored in saline solution-containing thymol granules following extraction. The extracted teeth bore no information regarding the patients' gender nor age. To streamline the calibration process and maximize space utilization by tooth samples, only three of the CSs, which best fitted the calibration function and covered the required

density range, were scanned with each sample. This enabled further voxel size reduction to achieve higher resolution. The three CSs (fluorite, selenite, and PET) with an extracted human tooth were placed in a vial, which was stabilized on the rotating mount of the CT-machine using sticky wax (Fig. 1b). The extracted tooth was kept humidified by immersion in saline solution and stabilized by compressing a piece of wax between its root and the tube's walls. A ROI containing the entire volume of enamel was created using the "surface determination" function and was refined with the "draw" tool to exclude the fluorite disc from the ROI since enamel and fluorite overlap in density. Under the Porosity/Inclusion Analysis module, a defect analysis was performed on the created ROI containing only enamel to obtain descriptive statistics of GSVs (Fig. 2). X-rays were generated at 110 kV and 160 μ A, creating 3,000 two-dimensional projections over a 360-degree rotation of the specimen with a voxel size of 7.1 μ m. Pre-filtration with a copper filter was used. Three-frame averaging was also applied. On average, the acquisition time was 158 min.

Results

The physically measured densities for the different CSs were PET ($\rho = 1.38 \text{ g/cm}^3$), acetal ($\rho = 1.41 \text{ g/cm}^3$), PPS ($\rho = 1.64 \text{ g/cm}^3$), selenite ($\rho = 2.24 \text{ g/cm}^3$), Egyptian alabaster ($\rho = 2.7 \text{ g/cm}^3$), aragonite ($\rho = 2.72 \text{ g/cm}^3$), and fluorite ($\rho = 3.11 \text{ g/cm}^3$).

Fig. 2. Defect analysis performed on created ROI containing only enamel. Descriptive statistics of GSVs were calculated for the entire volume of intact enamel. **a** Top-view workplace 2D window displaying defect analysis performed on extracted enamel (area colored red; color in online version only). Surface determination

function enabled us to define intact enamel and thus early enamel lesion present was excluded from analysis. **b** Same as **a**, but a front-view workplace 2D window. **c** Same as **a**, but a right-view workplace 2D window. **d** Same as **a**, but 3D window.

The examination of profile sections of the CSs confirmed the homogeneity and uniformity of the GSVs across the CSs. Figure 3a is a profile window of the fluorite disc. This graph illustrated the stability of GSVs across the distance drawn along the diameter of the disc and the negligible presence of beam hardening, as there are only minute fluctuations in the signal profile. The standard deviation measured for the created interval was 1,641 AU. Homogeneity and uniformity of the GSVs were also found for the remaining CSs.

To demonstrate the beam-hardening effect that would have been present without applying the extra measures to reduce it, a profile window was generated for the fluorite disc without applying a Cu filter nor beam-hardening correction module (Fig. 3b). The beam-hardening effect can be appreciated at the beginning and at the end of the distance measured. The standard deviation over the created interval was measured to be 4,232 AU. Figure 3c is a

profile window of the same fluorite disc, under the same settings, with Cu pre-filtration but without applying the beam-hardening correction module. The addition of a Cu filter reduced the beam-hardening effect. The standard deviation of the created interval in Figure 3c, 2,084 AU, was significantly less than the standard deviation in Figure 3b, 4232 AU.

Table 1 summarizes the descriptive statistics of the GSVs of the CSs derived from their representative volumes. The results showed that as the density of the material increases, the standard deviation of the associated GSVs also increases. Figure 4a is a scatter plot that displays the linear relationship between the mean GSV and the calculated density for the seven CSs. A squared Pearson correlation coefficient of $R^2 = 0.994$ was determined. The linear relationship was reproduced at different settings. Table 2 lists the regression data obtained, slope and intercept, at the different voltage set-

Fig. 3. Influence of pre-filtration and beam-hardening correction during reconstruction. **a** Profile window of fluorite disc scanned with radiation energy (110 kV, 160 μ A) with the application of both a Cu filter and the beam-hardening correction module during the reconstruction process. **b** Same as **a**, but without application of a Cu filter nor the beam-hardening correction module. **c** Same as **a**, but with Cu pre-filtration and without application of the beam-hardening correction module.

Fig. 4. Test of linearity between the mean GSVs versus physically calculated density. **a** Scatter plot of mean GSV versus calculated density for the seven CSs (A: PET; B: acetal; C: PPS; D: selenite; E: Egyptian-alabaster; F: aragonite; and G: fluorite). Squared Pearson correlation coefficient and calibration function are displayed. **b** Same as **a**, but for the three chosen CSs (A: PET; D: selenite; and G: fluorite).

tings. All R^2 values at the different voltage settings were >0.99 . A regression line drawn through best fitting the data points by the least squares regression equation method yielded a regression equation of $y = 4.00\text{E-}05x + 6.44\text{E-}01$. Figure 4b is a scatter plot that displays the linear relationship for the three chosen CSs. These three CSs yielded R^2 of 1. A regression equation of $y = 4.53\text{E-}05x + 6.13\text{E-}01$ was yielded.

The estimated density of the bovine enamel disc, based on the obtained calibration function, was 2.48 g/cm^3 . The physically measured density of the disc (7.63 mm in diameter, 1.19 mm in height, 0.13 g in weight) was 2.45 g/cm^3 . The calculated relative error was 1.2%. Densities of sound enamel in the 10 extracted human premolar teeth, based on the CT-GSVs, ranged from $2.6\text{--}3.1 \text{ g/cm}^3$.

Discussion

In this study, the CSs were chosen based on their densities, which ranged from $1.5\text{--}3 \text{ g/cm}^3$ to cover the anticipated density range of sound and carious enamel. As the main objective of this study was to develop an easy method of calibration, ready-made materials were chosen to eliminate the fabrication process of the traditionally used CSs. The examination profile sections of the CSs confirmed the homogeneity and uniformity of the GSVs and the effectiveness of the measures taken to minimize the beam-hardening effect across the seven CSs (Fig. 3a). Non-homogeneity results in variations in GSVs across the CS material, which may introduce errors in the estimation of the true mean GSV of the CS [24]. This might subsequently introduce error in density estimation using such method.

Consistent with previous studies [19, 25], our results showed that as the density of the material increases, the standard deviation of the associated GSVs also increases (Table 1). This can be attributed to the beam-hardening effect, scattering, and noise associated with higher-density materials such as fluorite. With the exception of PPS, the standard deviations of the remaining 6 CSs decreased from fluorite to PET in an expected manner. This is probably due to the fact that PPS, though homogeneous, was slightly granular. However, the fact that its mean GSV fitted the calibration curve led us to include it as a CS. Efforts should be directed at minimizing CT-scan artifacts, which in turn should reduce GSV variations within the material and ensure the reproducibility of GSV. It is worth noting that image de-noising techniques using known filters, such as Gaussian and median filters, were not used in this study. It was our intention to preserve the fine details of the images as the formerly mentioned techniques cause significant blurring of images with subsequent loss of fine details. The use of the “block-matching and three-dimensional method,” a method that has been shown to preserve the texture and fine details, may have benefitted in de-noising the micro-CT images in our study [26].

An R^2 of 0.99 indicated a very high correlation between the mean GSV of each CS and its calculated density. Scans taken at different settings confirmed the reproducibility and stability of this linear relationship independent of the composition of the CS and the voltage applied (Table 2). The regression equation derived enabled us to predict the density of a material based on its average GSV. The CT-estimated density of bovine enamel disc showed high accuracy when compared to the physically measured value. The CT-estimated density of intact human enamel in extracted premolars correlated well with the reported values making this system of CSs valid for enamel caries research. The reported densities of sound enamel in the literature ranged between 2.4 and 3.1 g/cm³ [6–9, 15, 27, 28].

Potential errors in density measurement using the above calibration method might derive from different sources. The chemical composition of a material is one source. If the material of interest contains some heavy metals, it will probably result in higher CT-GSV than expected. Another source of error is the extrapolation of the calibration function to estimate an unknown material's density outside the density range of the CSs used. Each calibration function is specific to the individual scan and cannot be applied to other scans. Hence, CSs should be scanned with each sample to derive its spe-

Table 1. Physically measured densities and mean GSVs of CSs with standard deviations (SD)

CS	Density, g/cm ³	Mean GSVs (SD), AU
Fluorite	3.1	59,979 (1,576)
Aragonite	2.72	52,642 (1,239)
Alabaster	2.7	50,584 (973)
Selenite	2.24	41,634 (689)
PPS	1.64	26,629 (1,471)
Acetal	1.41	18,141 (443)
PET	1.38	17,294 (503)

Table 2. Regression data obtained at different voltage settings using the least squares regression equation method

Voltage	Slope	Intercept	R^2
60	5.01E-05	5.77E-01	9.97E-01
70	4.58E-05	5.51E-01	9.95E-01
80	4.22E-05	6.45E-01	9.95E-01
90	4.14E-05	6.26E-01	9.94E-01
100	4.17E-05	6.23E-01	9.93E-01
110	4.00E-05	6.44E-01	9.94E-01

cific calibration function. CT-scan-associated artifacts such as noise, ring artifacts, scattering, and beam-hardening effects, can also be a source of measurement errors, especially when high-contrast resolution is required. Beam hardening produces a nonlinear signal-to-density relationship, which can affect the accuracy of micro-CT measurements [29]. This effect is more pronounced with higher-density materials. Typical measures to reduce it were employed by applying the beam-hardening correction module, pre-filtration, and through the automatic beam-hardening correction. Ring artifacts were reduced through flat-field correction [30]. To increase the signal-to-noise ratio and to decrease the noise of CT projections, frame averaging was applied. Image de-noising, using the block-matching and three-dimensional method, should be explored in future research related to the study of early enamel lesions. Despite all efforts made to reduce artifacts, the presence of some artifacts is inevitable, and if these artifacts were present at areas of interest, then they will undoubtedly affect measurements.

Conclusion

In the current study, seven minerals and polymers were found to be suitable CSs to establish a linear, reproducible calibration function covering the anticipated density range for enamel caries research. Fluorite, selenite, and PET, which best conformed to the calibration function, were chosen as a streamlined calibration set. With copper pre-filtration, beam-hardening correction during reconstruction, frame averaging, and flat-field correction, the derived calibration function accurately predicted the density of enamel based on its average GSV. The developed system of CSs with its derived calibration function is a simple, valid, and reproducible method to quantitate enamel mineral density for caries research.

Acknowledgements

The authors thank Rudolf Kusy, Shaji Michael, Sreeja Saji, and Merin Lejoe for their technical support.

Statement of Ethics

Ethical clearance was obtained from Kuwait University/Health Sciences Centre Ethical Clearance Committee (Ref.: VDR/EC/3332).

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

The project was funded by Kuwait University (Grant No. SRUL01/14).

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