European Review for Medical and Pharmacological Sciences 2023; 27 (3 Suppl): 61-70

Optical coherence tomography application for assessing variation in bone mineral content: a preclinical study

S. KHIJMATGAR[1,2], S. PANDA[3], R. BIAGI[2], M. ROVATI[2], L. COLLETTI[2], F. GOKER[2], A. GRECO LUCCHINA[4], C. MORTELLARO[4], M. DEL FABBRO[2,5]

[1]Queen Mary University of London, London, United Kingdom
[2]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy
[3]Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Siksha O Anusandhan (Deemed to be) University, Bhubaneswar, Odisha, India
[4]Research Laboratory in Regenerative Medicine and Tissue Engineering, Saint Camillus International University of Health Sciences, Rome, Italy
[5]Maxillo-Facial Surgery and Dental Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

C. Mortellaro and M. Del Fabbro share the last position

Abstract. – OBJECTIVE: Optical Coherence Tomography (OCT) is a non-invasive imaging technique that produces cross-sectional images through biological tissues, allowing three-dimensional reconstruction and analysis. Aim was to evaluate if OCT may discriminate among tissues with different bone density and composition, by measuring the depth of light penetration in porcine and rat bone samples.

MATERIALS AND METHODS: Two carpal bone samples (2 cm length) were harvested from the porcine forelimb and fixed overnight in 3.7% buffered formal saline. Following fixation, one sample was decalcified in a 1:1 mixture of 8% hydrochloric acid and 8% formic acid solution for three days, with solution changes each day. Samples were imaged using an OCT microscope. Furthermore, the calvaria, ulnar, alveolar and basal bone of mandible of 6 male and 6 female rats were cleared of overlying soft tissues and scanned under OCT. The light penetration depth in each sample was measured using the software Image J, and Scattering Attenuation Microscopy.

RESULTS: In the mineralized bone the average depth (μm) and standard deviation (SD) of light penetration were 790.1±18.05 and 410.4±21.7 for periosteal and endosteal surface, respectively, and 507.3±21.03 for cross-section surfaces, while it was 858.4±32.03 for periosteal surface, 1150±26.9 for endosteal, and 627.3±31.8 for cross-section bone surfaces in demineralized porcine bone. There was a significant difference ($p<0.001$) in depth of light penetration between normal and de-mineralized bone for all regions evaluated. No systematic significant difference in light penetration depth between-gender was found at any site evaluated, while there were variations between sites ($p<0.001$). The OCT detected differences in bone mineral and porosity among gender ($p<0.0001$)

CONCLUSIONS: This study suggests that OCT may represent a valuable technique to estimate local variations in bone mineral content.

Key Words:
Optical coherence tomography, Bone mineral density, Bone quality, Osteoporosis.

Introduction

The basic functions of bone are protection, weight-bearing, and mineral homeostasis. While humans start getting older, the porosity of bone increases leading to osteoporosis. Osteoporosis is characterized by the decrease in bone density or bone mineral content leading to an increased risk of fracture. In osteoporosis, bone mineral density (BMD) is reduced, and bone micro-architecture disrupted, and the amount and variety of non-collagenous proteins in bone is altered[1,2]. The development of a non-invasive techniques to quickly assess early changes and loss in bone mineral density in regions of the skeleton would be advantageous.

Current techniques to assess BMD include Dual energy X-ray absorptiometry (DEXA), va-

Corresponding Author: Massimo Del Fabbro, MSc, Ph.D; e-mail: massimo.delfabbro@unimi.it

rious types of computed tomography (CT), such as quantitative CT (QCT), peripheral quantitative CT (pQCT), high-resolution peripheral quantitative CT (HR-pQCT), quantitative ultrasonography (QUS)[2,3]. Each technique has benefits and disadvantages. DEXA has minimal radiation, high precision, reproducibility, and good correlation with fracture risk, but can be affected by many artefacts such as previous fractures, spinal disease, and obesity. QCT allows selective measurement of trabecular and cortical bone and gives true volumetric BMD, but delivers high radiation dose, and is less reproducible and standardized than other techniques. pQCT and HR-pQCT deliver minimal radiation. The former requires machines specifically designed for distal bone sites (usually radius or tibia), and its use is mostly limited to children. The latter is a non-invasive method of viewing three-dimensional microarchitecture and trabecular and cortical structure, but is currently very expensive, and is also dedicated to small peripheral regions only. Benefits of QUS include no ionizing radiation, and portability of the machine. This method calculates bone stiffness as a surrogate for bone density. Its limitations include significant manufacturer and operator differences. Ultrasound is not currently recommended for screening of osteoporosis.

Optical coherence tomography (OCT) is a non-ionizing, non-invasive three-dimensional imaging technique which produces cross-sectional images through biological tissues[4-6]. The principle is based on optical interferometry technique, which depends on interference between a split and later recombined broadband optical field[7]. It captures two dimensional cross-sectional images which are obtained from three dimensional samples. Its use in detecting the lesions at earliest stages in dentistry has being reported[8]. By using a low coherence interferometry (LCI) signal, hard tissues like enamel, dentin and dentin-enamel junction were studied and it was found that OCT could be used to quantitatively monitor the mineral changes[9]. The mineralization status and the scattering properties of the dental materials can be detected by the polarisation-sensitive OCT (PS-OCT)[10]. OCT has been also used to determine the mineral content in bone studies[11]. There are various modalities in detecting bony abnormalities, but to detect the earliest stages of osteoporosis with an imaging tool would benefit both patients and health care providers.

The study aims were: a) to determine the accuracy of optical coherence tomography (OCT) technique in measuring the variations in bone mineral density of normal and demineralized porcine metacarpal bone samples and b) to determine whether OCT light penetration depth could be used to discriminate between gender and site specific in different bone types in a rat model.

Materials and Methods

To address the first objective, two carpal bone samples of 2 cm length from the porcine forelimb were obtained from a butcher's shop, so ethical approval was not needed.

For the second objective, male (n=6) and female (n=6) rats of equal age (euthanized by CO_2 before scanning under OCT. The rats were obtained from QMUL, animal house and the animal care followed the UK animal house regulations. The euthanization procedure was based on animal house regulations of Queen Mary University of London (QMUL), UK (https://www.qmul.ac.uk/research/animal-research/) and was carried out by animal technician at animal house, QMUL. The animals were control rats discarded from other studies. Therefore, specific ethical approval was not needed.

Tissue Preparation

Porcine carpal bone samples were cleared of overlying soft tissues and fixed overnight in 3.7% buffered formal saline. A standard method of demineralization was followed using 8% hydrochloric acid and formic acid. Later, the samples were cut in longitudinally to expose endosteal surface[12].

The samples from rats were denuded of soft tissue and only bone was scanned under OCT. Bone samples were used to quantify differences in depth of light penetration in various functional and anatomical skeletal sites. The calvaria, ulnar, alveolar, and basal bone of mandible of the 12 rats were cleared of attendant soft tissues and scanned under OCT (sites highlighted in **Supplementary Figure 1A-C** and **Supplementary Figure 2A-C**).

Imaging Technique Description

Samples were imaged using an OCT microscope (EX1301, Michelson Diagnostics Ltd, UK) operating at a central wavelength of 1300 nm with

axial and lateral resolutions of approximately 10 and 9 μm respectively using a medium of water to avoid dehydration of bone during scanning. OCT measures the backscattered light generated from an infrared light source as a function of depth. It uses LCI to produce microscopic cross-sectional images of tissues similar to histology. OCT system which can collect A-scans (depth versus reflectivity curve), B-scans (longitudinal images) and C-scans (transverse images at constant depth) was used. OCT image volumes perpendicular to the long axis (3×3×1.5 mm) were obtained from the periosteal, endosteal surfaces, and measurements taken from the exposed cut surface (parallel to the longitudinal axis) from the normal and demineralized samples, and the images were analyzed using Image J software (Version 1.52, National Institute of Health, Bethesda, MD, USA).

The B-scans (cross-sectional image where the amplitudes of reflections are represented in a grey scale or a false-color scale)[9] were obtained, consisting of alternate bands wherein they are wavy in nature, resulted from repeated stoppage of light penetration.

The penetration depth of light was determined by scattering attenuation microscopy (SAM software)[13,14].

Methods of Quantifying Light Penetration Depth

Manual method

The manual method is carried out by processing the OCT images in Image J software. The initial image obtained is altered by changing the color threshold and contrast with the help of ImageJ software, to highlight approximate penetration depth of light and each depth is measured using free hand lines and choosing approximately equal distances of 1-2 mm away from each measurement.

Sam software

Scattering Attenuation Microscopy (SAM) works on the principle of spectral domain low coherence interferometry (LCI)[13]. The SAM image depends upon the acquisition of volumetric datasets. Each depth profile within the volume, the gradient of signal loss or attenuation is measured and represented within a color coded two-dimensional quantitative image of the dataset. This data set is used to plot the histograms from the SAM images and the type of distribution is studied for both normal and demineralized sections of bone samples.

Statistical Analysis

The values obtained for each section of normal and demineralized bone were tabulated and results are presented as mean values (μm), standard deviation (SD), standard error (SE). The sample sites of normal and demineralized sites were compared utilizing the students' *t*-test. The values obtained were used for plotting bar chart. Multiple Variance Tests (ANOVA) was conducted to determine the differences and variations in mineral content of male and female rats in each region evaluated. When the data detected to be not normally distributed, non-parametric test was performed (Wilcoxon signed rank test). A value of $p<0.05$ was considered as being statistically significant.

Results

Mineralized vs. Demineralized Bone

The results obtained from the preliminary analysis by manual method of the porcine carpal bone samples demonstrated a significant difference between the depths of light penetration in mineralized versus demineralized bone.

Mineralized Bone

The average depth (μm) ± 1 SD of light penetration into the periosteal surface of normal porcine bone was 790.1±18.1 μm, into the endosteal surface it was 410.4±21.7 μm, and for cross-section it was 507.3±21.0 (Figure 1).

Demineralized Bone

Similarly, light penetration into demineralized porcine bone averaged 858.4±32.0 μm for periosteal surface, 1150±26.9 μm for endosteal, and 627.3±31.8 μm for cross-section bone surfaces (Table I). The bar graph in Figure 1 shows that; the penetration of light in demineralized bone is greater on endosteal surface than in the cross-section.

The depth of light penetration in periosteal bone, endosteal bone and cross-section was statistically significant ($p<0.001$) (Table II and III) and when compared with demineralized with mineralized bone the depth of light penetration was significant (Table I) ($p<0.001$).

Scattering Attenuation Microscopy (SAM)

Figure 2A and 2B illustrate the scans from the optical coherence tomography, followed by Figure 2C and 2D SAM images illustrating the depth of light penetration into the periosteal sample. There was varying penetration of light into the sample. The linear values were represented in the form of histogram (Figure 2E).

Similarly, Figure 3A and 3B illustrate the scans from optical coherence tomography, followed by Figure 3C and 3D SAM images that illustrate the depth of light penetration into the endosteal surface of the normal and de-mineralized bone. The depth of light penetration was more in Figure 3D. The linear values from SAM imaging represented in the histogram (Figure 3E).

Effect on Gender and on Bone Region

The differences in depth of light penetration in different rat bone regions were demonstrated in the Figure 4. There are no statistically significant differences at any specific sites between the genders, but the histogram figure showed that there is a spread of means between the sites (Figure 4).

In Table IV the differences and variations in mineral content of male and female sites (p<0.0001) are presented. There was a significant difference between left mandible (basal) and left mandible (alveolar) (p=0.012). There was a significant difference between calvaria (endosteal) *vs.* right mandible (basal, p=0.012) and left mandible (basal, p=0.018). Finally, there was a significant difference between sites of right mandible (basal) and left mandible (alveolar) (p=0.018) (Table IV).

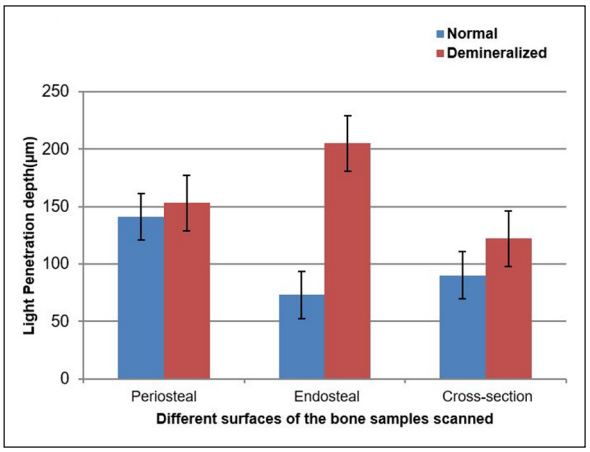

Figure 1. Depth of Light Penetration in normal and demineralized bone sample from different surfaces (Image J Software measurements).

Discussion

The National Institute of Health defined bone quality as the material, architectural and mechanical characteristics with which bone mass contribute to bone strength. The porosity of cortical bone ranges between 5% and 10% and that of trabecular bone is 50% to 90%[11]. There are different imaging modalities employed to detect the tissue composition, geometry and micro-architecture. DEXA scan is the current gold standard modality use to check the geometry and micro-architecture[15]. Another technique for direct measurement of bone tissue mechanical behavior (bone quality) is mechanical indentation[16]. These techniques use ionizing radiation and invasive procedures.

Table I. The mean, standard deviation, standard error, *t*-test for normal and demineralized porcine bone samples and percentage change in light penetration depth.

Sample sites	Normal	Demineralized	*t*-test	% change
Periosteal Surface			< 0.001	7%
Average mean (µm) ± SD	790.1 ± 18.05	854.4 ± 32.03		
SE	2.1	3.3		
Endosteal Surface			< 0.001	64%
Average mean (µm) ± SD	410.4 ± 21.7	1150.8 ± 26.9		
SE	2.1			
Cross-Section			< 0.001	19%
Average mean (µm) ± SD	507.3 ± 21	627.3 ± 31.8		
SE	2.9	4.4		

Table II. Mineralized bone sample surfaces. The table shows the significance when compared with different sites and surfaces of normal bone samples.

Site	Periosteal	Endosteal	Cross-section
Periosteal	-	xx	xx
Endosteal	xx	-	xx
Cross-section	xx	xx	-

x: Not significant, xx: Significant, xxx: Highly significant.

Table III. Demineralized bone sample surfaces. The table shows the significance when compared with different sites and surfaces of normal bone samples.

Site	Periosteal	Endosteal	Cross-section
Periosteal	-	xx	xx
Endosteal	xx	-	xx
Cross-section	xx	xx	-

x: Not significant, xx: Significant, xxx: Highly significant.

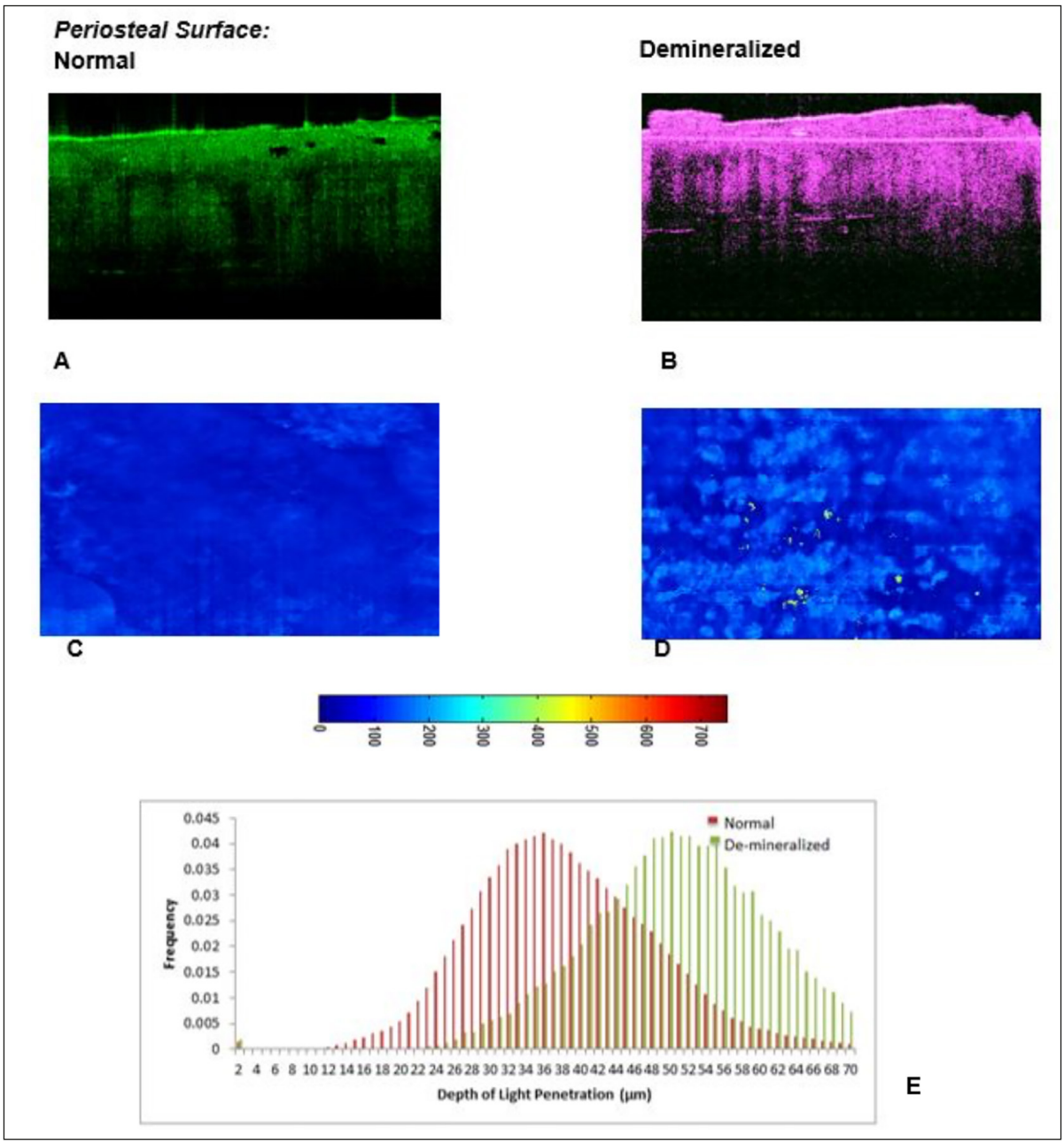

Figure 2. Scattering Attenuation Microscopy (SAM) of porcine limb bone samples. **A,** Normal, (**B**) Demineralized, (**C**) SAM image of normal bone with lesser penetration of light and (**D**) SAM image of the de-mineralized with greater penetration of light. **E,** The linear values were represented in the form of histogram.

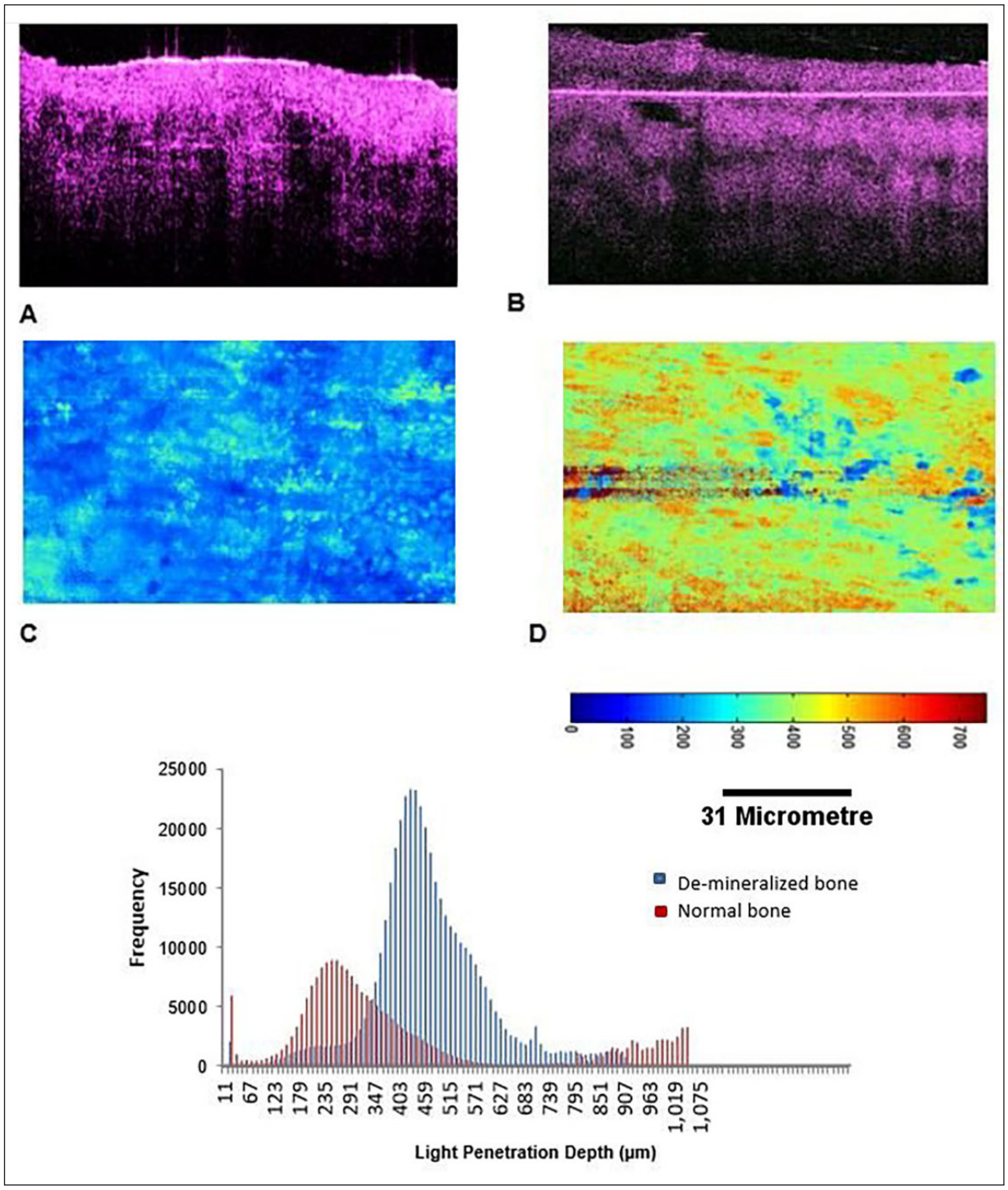

Figure 3. Scattering Attenuation Microscopy (SAM) of porcine limb bone samples. **A,** OCT image of endosteal surface of normal bone. **B,** Endosteal surface of demineralized bone showing greater penetration into the sample compared to endosteal surface of normal bone. **C,** There is least penetration of light in to the normal endosteal surface of bone. **D,** There is larger penetration of light into the demineralized surface of the bone. The histogram is showing the separation between the two means and the data is widely distributed endosteal surface of normal and demineralized bones. **E,** The linear values from SAM imaging represented in the histogram.

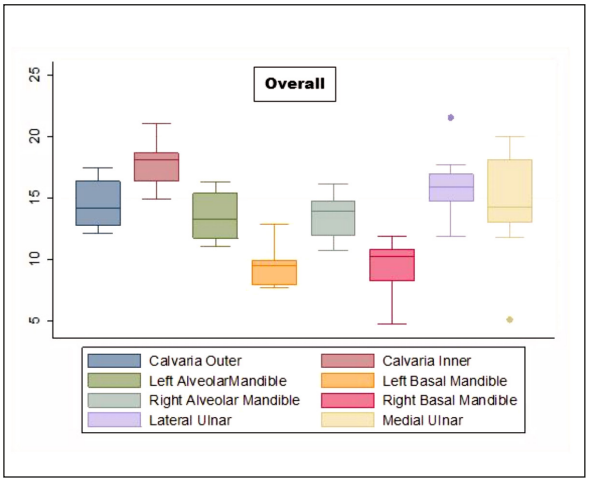

Figure 4. Box plot for depth of light penetration in different sites of rat bone samples (Y axis: Depth of light penetration).

OCT is a novel modality which uses non-ionizing, non-invasive and produces three dimensional images which can detect bone changes at its earliest stages[16-18].

The results of this study show that the penetration of broadband light was greater in demineralized than in normal bone samples. There was a significant difference between various regions in the mineralized sample versus the demineralized sample i.e., the degree of penetration was greater in periosteal, endosteal and cross-sectional surfaces of demineralized than in normal (mineralized) bone (Table II and III). The penetration was greater into the endosteal surface of the demineralized bone sample than either the periosteal or cross-section surface of normal bone (Figure 1). The greater penetration was due to greater porosity that enable the light go deeper depth into the sample. Lesser the porosity and more dense bone, lesser the depth of light penetration. It is seen that there was a 64% change in penetration of light into the endosteal surface between normal and demineralized samples. The means and standard deviations given in Table I indicate significant difference between normal and demineralized bone. This significant result may be due to the loss of bone minerals and decrease in bone mineral density from the bone sample after demineralization (Table II and III). The reason for difference in depth of light penetration is due to least scattering of light from the samples, which contains less mineral content. The difference in the penetration of light in the sample was found to be 7% change for periosteal surface, and 19% change from cross-section.

Scattering attenuation microscopy (SAM) uses SAM software by generating volumetric data and SAM images, showing the depth of penetration of light from the various sites and surfaces. The SAM method was followed to validate the results obtained from the manual method. The OCT images of the porcine bone samples are processed in SAM software (Figure 2 and 3). Preliminary results of all SAM images from a porcine normal and demineralized bone samples revealed significant differences. The SAM image of periosteal surface of the demineralized bone sample (Figure 4) shows the amount of penetration was greater than into normal bone sample (Figure 2). Similarly, the depth of light penetration was greater in demineralized bone (Figure 2 and 3). The histogram was plotted to identify the spread of data along the means in normal distribution. There is wide separation along the means, and this data shows the OCT can detect changes in the bone mineral content (Figure 3). Its principle of working is explained based on the polarized light scattering.

In bone and tooth, anisotropy is due to mineralized structures originating from hydroxyapatite nanocrystals, which play an important role in hard tissue depolarization and birefringence. Since OCT may detect small changes in mineral content, it may be helpful to identify early mineral loss from the hard tissues, and possibly to take preventive measures before such changes become pathological.

Similarly, there were differences observed in male and female rat bone samples (Figure 4). Physiologically, the bone is less dense in females as compared to that of males[19,20]. Our results suggest that the OCT technique may represent a feasible method for differentiate bone density in male and female, as a function of depth of light penetration. Further development of algorithms that enable greater penetration into the tissues through the skin would be added advantage to the current systems.

The main limitation of the study is the reduced sample size, which was due to a reduced budget. In spite of this, interesting outcomes were found, however this needs to be confirmed by subsequent studies with a wider sample size. Furthermore, future studies performed in human model are necessary to support results obtained in animal models, in which bone architecture and mineralization pattern may differ from that of humans.

Table IV. Significance of the Multiple Variance Tests (ANOVA) to determine the differences in mineral content.

Site		*p*-value
Calvaria (Periosteal)	Calvaria (Endosteal)	0.994
	Left Mandible (Alveolar)	0.702
	Left Mandible (Basal)	0.059
	Right Mandible (Alveolar)	0.978
	Right Mandible (Basal)	0.086
	Ulnar (Lateral)	0.993
	Ulnar (Medial)	1.000
Calvaria (Endosteal)	Calvaria (Periosteal)	0.994
	Left Mandible (Alveolar)	0.288
	Left Mandible (Basal)	0.012*
	Right Mandible (Alveolar)	0.701
	Right Mandible (Basal)	0.018*
	Ulnar (Lateral)	0.797
	Ulnar (Medial)	0.976
Left Mandible (Alveolar)	Calvaria (Endosteal)	0.702
	Left Mandible (Alveolar)	0.288
	Left Mandible (Basal)	0.826
	Right Mandible (Alveolar)	0.996
	Right Mandible (Basal)	0.897
	Ulnar (Lateral)	0.985
	Ulnar (Medial)	0.825
Left Mandible (Basal)	Calvaria (Endosteal)	0.059
	Left Mandible (Alveolar)	0.012*
	Left Mandible (Basal)	0.826
	Right Mandible (Alveolar)	0.389
	Right Mandible (Basal)	1.000
	Ulnar (Lateral)	0.295
	Ulnar (Medial)	0.098
Right Mandible (Alveolar)	Calvaria (Endosteal)	0.978
	Left Mandible (Alveolar)	0.701
	Left Mandible (Basal)	0.996
	Right Mandible (Alveolar)	0.389
	Right Mandible (Basal)	0.488
	Ulnar (Lateral)	1.000
	Ulnar (Medial)	0.995
Right Mandible (Basal)	Calvaria (Endosteal)	0.086
	Left Mandible (Alveolar)	0.018*
	Left Mandible (Basal)	0.897
	Right Mandible (Alveolar)	1.000
	Right Mandible (Basal)	0.488
	Ulnar (Lateral)	0.382
	Ulnar (Medial)	0.139
Ulnar (Lateral)	Calvaria (Endosteal)	0.993
	Left Mandible (Alveolar)	0.797
	Left Mandible (Basal)	0.985
	Right Mandible (Alveolar)	0.295
	Right Mandible (Basal)	1.000
	Ulnar (Lateral)	0.382
	Ulnar (Medial)	0.999
Ulnar (Medial)	Calvaria (Endosteal)	1.000
	Left Mandible (Alveolar)	0.976
	Left Mandible (Basal)	0.825
	Right Mandible (Alveolar)	0.098
	Right Mandible (Basal)	0.995
	Ulnar (Lateral)	0.139
	Ulnar (Medial)	0.999

*Significant difference.

Conclusions

The OCT technique can identify differences and variations in light path into bone, reflecting the degree on mineral content in normal and demineralized porcine metacarpal bone samples. There were differences in light path penetration in rat bones of different gender at all sites tested. OCT technique is sensitive enough to determine major differences in bone mineral content. Whether OCT can be a potential novel non-ionizing and non-invasive modality to assess risk of osteoporosis requires further study.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

None.

Ethics Approval

Ethics approval was not necessary because it was a laboratory study, that used tissues either purchased from a butcher's shop (porcine bone), or animals (rats) discarded from other experiments, that were euthanized following the UK animal house regulations.

Funding Statement

The authors received no specific funding for this work.

Authors' Contribution

S.K., S.P., R.B., M.R., L.C., F.G., A.G.L., C.M. and M.D.F. conceived and designed the analysis. All the authors contributed on analysis and interpretation of data for the work. All authors revised the work critically for intellectual content. Integrity of the work was appropriately investigated and resolved by all authors. All authors contributed and approved equally to the final version of the manuscript.

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