Abstract: Osteoporosis is a prevalent bone condition, characterised by low bone mineral density and increased fracture risk. Currently, the gold standard for identifying osteoporosis and increased fracture risk is through quantification of bone mineral density (BMD) using dual energy X-ray absorption (DEXA). However, the risk of osteoporotic fracture is determined collectively by bone mass, architecture and physico-chemistry of the mineral composite building blocks. Thus DEXA scans alone inevitably fail to fully discriminate individuals who will suffer a fragility fracture. This study examines bone at both ultrastructural and microarchitectural levels to provide a detailed material view of bone, and therefore produce a more comprehensive model of osteoporotic fracture risk. Physico-chemical characterisation obtained through X-ray diffraction and infrared analysis indicated significant differences in apatite crystal chemistry and microstructure between fracture and non-fracture groups. Further, this study, through considering the potential correlations between the chemical biomarkers and microarchitectural properties of the bone, has investigated the premise that bone mechanical properties (e.g. fragility) are affected by physicochemical material features.
**Highlights**

- Physicochemical properties assessed through XRD and FTIR for non-fracture and fracture human specimens.
- Significant differences in coherence length, ‘a’ axis lattice parameters and carbonate: phosphate ratios between the two groups.
- With age, an increase in the phosphate to amide ratio and the coherence length was observed.
- Mineral properties of bone at the ultrastructure level may influence the micro architectural properties of trabecular bone.
- Possible differences in the material quality between fracture males and females, as observed through tissue mineral density (TMD) values.
Towards New Material Biomarkers for Fracture Risk


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Abstract

Osteoporosis is a prevalent bone condition, characterised by low bone mineral density and increased fracture risk. Currently, the gold standard for identifying osteoporosis and increased fracture risk is through quantification of bone mineral density (BMD) using dual energy X-ray absorption (DEXA). However, the risk of osteoporotic fracture is determined collectively by bone mass, architecture and physico-chemistry of the mineral composite building blocks. Thus DEXA scans alone inevitably fail to fully discriminate individuals who will suffer a fragility fracture. This study examines bone at both ultrastructure and microarchitectural levels to provide a detailed material view of bone, and therefore produce a more comprehensive model of osteoporotic fracture risk. Physico-chemical characterisation obtained through X-ray diffraction and infrared analysis indicated significant differences in apatite crystal chemistry and microstructure between fracture and non-fracture groups. Further, this study, through considering the potential correlations between the chemical biomarkers and microarchitectural properties of the bone, has investigated the premise that bone mechanical properties (e.g. fragility) are affected by physicochemical material features.

Keywords: aging, bone quality, hydroxyapatite, osteoporosis, trabecular bone, X-ray diffraction
Introduction

Osteoporosis affects approximately 200 million women around the world. In the UK alone 50% of women will suffer a fracture after the age of 50 [1], a rate which is annually increasing due to the aging population. Osteoporotic fractures often occur in the hip, wrist and vertebrae; although studies have shown hip fractures have the greatest detrimental effect on an individual [2]. Hip fractures result in a significant loss of independence, and sufferers are unable to live without support as they cannot walk unaided or perform many of their daily activities. Worryingly, hip fractures are often associated with increased mortality [3,4], a statistic which is confounded by the asymptomatic nature of osteoporosis. Osteoporosis is often assessed according to an individual’s bone mineral density (BMD) [5]. With a decrease in BMD, the risk of fracture is significantly increased [6]. Currently the gold standard for measuring BMD is through the use of dual energy X-ray absorption (DEXA). Unfortunately this method is a poor predictor of fracture, with a study carried out by Wainwright et al. showing that 54% of new hip fractures occurred in women who did not have osteoporosis as determined by their BMD [7] and data from the National Osteoporosis Risk Assessment, showed that 82% of post-menopausal women with fractures had bone of 'normal' BMD [8].

The limitations associated with BMD to predict an individual patient’s fracture risk is arguably because it does not measure the multiple material factors that contribute to bone strength [9]. Bone strength is a combination of bone density as well as ‘bone quality’, whereby bone quality refers to bone architecture (i.e. macro and micro) and bone chemistry [9]. A small number of studies (possibly due to the difficulty of obtaining human bone, especially osteoporotic specimens) have shown microarchitectural properties of bone potentially offer a superior way to differentiate between diseased bone (fractured due to osteoporosis or osteoarthritis) when compared to healthy controls (non – fractured tissue) [10 - 12]. Bone chemistry is more complex, with studies often providing contradicting results and conclusions [13 – 18]. Unfortunately, many of the studies which investigate the chemistry of osteoporotic bone are limited by relatively low sample numbers (n < 6 for both osteoporotic and ‘normal’ specimens) [16, 19, 20] and/or utilise ovariectomised animal models [21, 22]. A more recent study by Boskey [15], investigated the material properties of a large number of cortical and trabecular specimens (n = 120) using Fourier transform infrared spectroscopy (FTIR). However, the bone specimens were collected from the iliac crest (as a proxy for fractures at other sites) between 6 months and 5 years after a fracture and such a delay may
be confounding due to the complexities of remodelling and changes to the bone through aging. Not surprisingly then, the results of many previous studies tend to be conflicting.

Several previous studies have examined the physicochemical properties of the inorganic bone component (i.e. the hydroxyapatite mineral) characterised by X-ray diffraction (XRD) [13, 19, 20] and the organic component (i.e. collagen) as characterised by Raman spectroscopy [23, 24] or Fourier transform infra-red spectroscopy (FTIR) [21, 25, 26]. For example, a recent study of three samples (per group, normal, osteopenic and osteoporotic) by Rollo et al suggested a decrease in crystallite size and an increase in lattice microstrain in osteoporotic trabecular bone compared to ‘normal’ specimens [20]. Satry et al. also also reported a decrease in crystallite size in osteoporotic and overiectomized bone tissue [19]. In contrast, reports such as those of Thompson et al [13] and Faibish and Boskey [27] suggested an increase in crystallite size in osteoporotic tissue. These two reports differ however in conclusions regarding the crystal chemistry; Thompson suggesting a decrease in carbonate [13], while Faibish and Boskey [27] argues there is an increase. An increase in both crystallite size and carbonate content was reported by Gadeleta et al [14]. Several reports have suggested that there is no significant difference between osteoporotic and normal bone tissue when considering crystallite size [16, 22, 28]. Although a review by Boskey in 2003 reported that the general consensus accepts that osteoporotic bone mineral has significantly larger crystallites than the non-osteoporotic counterparts [15], it is evident from the literature this view point is contentious. A more recent study by Boskey et al. [18] reported a significant decrease in carbonate to phosphate ratios in that of fractured bone compared to non-fractured cortical bone, suggesting either a decrease in carbonate and/or an increase in phosphate. No other significant differences were observed for either cortical or trabecular bone. In contrast, McCreadie et al. reported an increase in the carbonate to phosphate ratio between specimens collected from women with and without osteoporotic fractures [23].

Very few studies have examined changes to the hydroxyapatite unit cell parameters (as a proxy for substitutional modifications) of osteoporotic and/or aged bone mineral [28, 29]. The major substitution in biological hydroxyapatite is carbonate, which substitutes for the hydroxyl (A – type) and/or phosphate (B-type) in the crystal lattice or exists on the apatite surface (labile carbonate) [30, 31]. In general, a decrease in ‘a’ axis and an increase in the ‘c’ axis lattice parameters has been reported with age [29]. These trends have previously been associated with an increase in B-type carbonate substitution [30]. In contrast other studies were unable to detect differences in the lattice parameters of osteoporotic bone [28]. As a
further bone characteristic measured by FTIR, it has been reported that for osteoporotic
tissues the mineral to organic ratio is significantly lower than that of normal bone [14, 25].

This study reports the physicochemical properties assessed using XRD and FTIR for
trabecular bone obtained from the femoral head of individuals who suffered a femoral neck
fracture and from a corresponding group where no fracture was reported. Further to this
investigation, the data provided an opportunity to explore relationships between the
ultrastructure material building blocks and the derived architectural properties. Thus for the
first time, the potential effect of the physicochemical properties on the micro architectural
properties of bone was investigated. This component of the work only involved the fracture
group as relatively large deviations in architecture would be expected in this group.

Materials and Methods

Bone Specimens

A sample set of 20 femoral heads were collected from osteoporotic female patients who had
suffered trauma fractures at the femoral neck and consequently required hip replacement
surgery. Of these 20, the donor’s age was available for 16 of the femoral heads. Ethical
approval for the collection and use of these specimens was provided by Gloucestershire NHS
trust REC. Non-fracture femoral head specimens were collected from 39 female donors
within the Melbourne Femur Collection. All donors from this source were coronial cases and
had therefore died suddenly and unexpectedly as result of accidents. Ethical approval for the
collection and use of these specimens was provided by Melbourne University. Population
characteristics for both fracture and non – fracture specimens are provided in Table 1.
<table>
<thead>
<tr>
<th></th>
<th>Fracture</th>
<th>Non-Fracture</th>
</tr>
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<tbody>
<tr>
<td>Donors</td>
<td>16</td>
<td>39</td>
</tr>
<tr>
<td>Age Range (yrs)</td>
<td>59 - 91</td>
<td>20 – 90</td>
</tr>
<tr>
<td>Age Mean (yrs)</td>
<td>82.4 ± 6.4</td>
<td>66.1 ± 17.9</td>
</tr>
<tr>
<td>Weight Range (kg)</td>
<td>41 - 79</td>
<td>40 - 121</td>
</tr>
<tr>
<td>Weight Mean (kg)</td>
<td>61.1 ± 8.9</td>
<td>66.7 ± 19.7</td>
</tr>
<tr>
<td>Stature Range (cm)</td>
<td>155 - 173</td>
<td>145 - 169</td>
</tr>
<tr>
<td>Stature Mean (cm)</td>
<td>163.9 ± 5.2</td>
<td>159.6 ± 6.7</td>
</tr>
</tbody>
</table>

Table 1: Population characteristics for fracture and non-fracture groups. The standard deviations for all mean values are provided.

Wherein quantitative comparisons are made between groups (fracture and non-fracture), the samples are age matched (70 + years) to avoid any bias arising from differences in age distributions.

**Sample Preparation**

Sectioning from the femoral head has previously described in detail [32]. Prior to data collections, the specimens were homogenised using a Retsch mixer miller (mm 2000) and a zirconium oxide milling basket and ball. The specimens were cut into smaller sections, to reduce the number of milling cycles and milled for one minute. Between milling, the specimens were allowed to stand for approximately one minute before milling was continued. This limited the potential of the specimen ‘over heating’ during milling, which could potentially cause changes to the mineral microstructure. Once powdered, the specimens were sieved through a stainless steel mesh sieve of 106 μm to ensure a homogenous fine powder sample.

**X-ray diffraction (XRD)**

The powdered specimens were individually loaded on to low background scattering (off-cut silicon) XRD holders. The bone powder was spiked with a NIST standard silicon powder (REF) to provide an internal standard required for determining accurate lattice parameters.

XRD analysis was carried out using a PANalytical X’Pert PRO Multi-Purpose Diffractometer with Cu Kα radiation. A PIXcel strip detector was used to collect data as stepped scans across an angular range of 15 – 80 2θ (°) (5.90 – 1.20 Å). The count time at each step was equivalent
to ~ 1 second. Data was also collected for two further stepped scans under the sample conditions but across an angular range of 23 – 27 20 (°) (3.86 – 3.30 Å d-spacing) and 50 – 55 20 (°) (1.82 – 1.67 Å d-spacing), and with a count time at each step equivalent to ~ 3 seconds. The two additional stepped scans were collected to provide greater quality data for the 002 and 004 Bragg maxima respectively. This data was used to accurately calculate the full width half maximum (FWHM) of both the 002 and 004 Bragg maxima. The FWHM values were then used to calculate coherence length using the Scherrer equation, as described below. Bruker Topas software (Version 4.1, 2008) was employed to undertake profile fitting of each diffraction profile. This provided quantitative crystallite size and morphology parameters through calculation of the coherence length and structural parameters of the crystal lattice through the lattice parameters.

Coherence length was calculated for two orthogonal crystallographic directions, <00ℓ> and <0k0> using the Scherrer equation, which uses the instrument corrected, full width half maximum of the desired peak, as described in [33]. The lattice parameters were calculated from whole pattern fitting refinement of diffraction profiles to obtain the 2θ peak positions. Sample displacement was refined and lattice parameter data corrected accordingly. No unit cell content model was applied and analytical peak shapes were pseudo-Voigt. The data collection, correction and analyses were repeated three times for five randomly selected specimens to assess repeatability.

**Fourier Transform Infrared Spectroscopy (FTIR)**

FTIR analysis was carried out using an attenuated total reflectance – FTIR. ATR-FTIR reduces sample preparation as there is no requirement for preparation of potassium bromide (KBr) pellets and the bone powder can be examined directly. This reduces potential contamination. Approximately 2 mg was used for analysis and three repeats per specimen analysed. FTIR spectra were collected using a Bruker Alpha Platinum ATR and analysis carried out using PerkinElmer Spectrum software. A scan resolution of 4 cm\(^{-1}\) and 16 scans was employed for data collection, within a range of 2500 – 400 cm\(^{-1}\).

FTIR analysis was employed to provide semi-quantitative data on the organic and carbonate content in the specimens. Following convention [34 - 37], the organic: mineral ratio was assessed through measuring the area of the amide I (1750 – 1600 cm\(^{-1}\)) and the \(v_3\) phosphate (1200 – 900 cm\(^{-1}\)) bands. The carbonate: phosphate was assessed through measuring the area
of the $v_2$ carbonate (890 – 850 cm$^{-1}$) and $v_3$ phosphate bands. Three repeats per specimen were taken to assess repeatability.

**Micro Computed Tomography (µ - CT)**

It is hypothesised that correlations between the physicochemical and architectural properties would be more evident than in the non-fracture bone. The architectural properties were obtained from µ- CT, details of which can be found elsewhere [32]. Parameters such as trabecular number (TbN), structure model index (SMI), trabecular thickness (TbTh), bone volume to total volume (BV/TV) and tissue mineral density (TMD) were previously reported.

**Statistical Analysis**

Linear regression analysis was carried out to statistically assess correlations between various material characteristics parameters and age for the non-fracture group. A general linear model ANOVA analysis was also undertaken to determine significant differences between the parameters measured for age matched fracture and non-fracture groups. $p < 0.05$ was considered statistically significant for both the linear regression and ANOVA analysis.

**Results**

This study reports the material quality of trabecular bone from human fracture (n=16) and non – fracture specimens (n = 39), with the parameters analysed correlated to age. Further, the material quality parameters associated with the fracture specimens have been correlated to the microarchitecture properties (previously reported in [32]). This provides a more comprehensive understanding of how a change in the material chemistry can be associated with and perhaps influence parameters such as trabecular number (TbN), tissue mineral density (TMD), structure model index (SMI) and bone volume to total volume (BV/TV). The average values and associated errors of the material properties derived from both XRD and FTIR analysis are presented in table 2.
Table 2: Average values (in bold) and the associated errors (SEM) for the material parameters obtained from XRD and FTIR analysis, for fracture and non-fracture groups.

**X-ray Diffraction (XRD)**

Coherence length, which provides a quantitative estimate of crystallite size and strain combined (i.e. the total lattice disorder), did not change significantly with age for the non-fracture group in the <00ℓ> crystallographic direction, but was found to increase significantly (p = 0.016) with age when the <0k0> direction was considered (Figure 1A). The coherence length values for the fracture group were significantly lower (p = 0.036) when age matched to the non-fracture group specimens in the <00ℓ> direction (see Table 3). With increasing age, the lattice parameters associated with the ‘a’ axis and ‘c’ axis remain, within experimental errors, constant for the non-fracture group, ranging from approximately 9.40 – 9.41 Å for the ‘a’ axis (Figure 1B) and 6.85 – 6.90 Å for the ‘c’ axis. The ‘a’ axis lattice parameter values for the fracture group are significantly less (p = 0.001) than those of the non-fracture group (see Table 3). No significant difference in the ‘c’ axis lattice parameters were observed between the two groups. Note that for characteristics of the non-fracture group that showed no significant age dependence, similar levels of significance were observed when all specimens were included within the ANOVA analyses.
Figure 1: Relationship between X-ray diffraction parameters and age (A: coherence length (CL) values along the 00ℓ direction vs age, B: ‘a’ axis lattice parameters vs age), for fracture and non-fracture specimens. Errors have been excluded from the graphs for clarity.
<table>
<thead>
<tr>
<th>Linear Regression Analysis</th>
<th>ANOVA</th>
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<tbody>
<tr>
<td>Non - Fracture Correlations with Age</td>
<td>Non - Fracture vs Fracture (Age Matched)</td>
</tr>
<tr>
<td>p - value</td>
<td>R²</td>
</tr>
<tr>
<td>CL₀₀₀f (nm)</td>
<td>0.928</td>
</tr>
<tr>
<td>CL₀₀₀₀ (nm)</td>
<td>0.016</td>
</tr>
<tr>
<td>LP 'a' axis (Å)</td>
<td>0.390</td>
</tr>
<tr>
<td>LP 'c' axis (Å)</td>
<td>0.207</td>
</tr>
</tbody>
</table>

Table 3: *Left:* p – values and R² calculated from linear regression statistical analysis when comparing the various XRD material characteristic parameters and age for the non-fracture group. For the parameters were a significant trend was observed, the rate of change (Δ) per 5 years is also reported. *Right:* 3: p – values for age matched ANOVA analysis of fracture (n = 15) and non-fracture groups (n= 22), for each XRD material characteristic parameter. The mean difference between the non-fracture and fracture groups is also reported, for those parameters found to be significantly different (p > 0.05).

**Fourier Transform Infrared Spectroscopy (FTIR)**

The carbonate to phosphate ratio was found to be significantly greater (p = 0.013) for the fracture group in comparison to the non-fracture group, when age matched. No age related trends were observed for the non – fracture group (Figure 2A). The phosphate to amide ratio values, which indicate an increase in collagen and/ or a reduction in phosphate, are presented in figure 2B for both fracture and non – fracture groups. The variability in the phosphate to amide ratio for the non – fracture group is evident. When age matched, the phosphate: amide values are not significantly different (p = 0.403) for the fracture group in comparison to the non – fracture group. However, with age, an increase in phosphate to amide ratio values was observed (p = 0.023) for the non-fracture group.
Figure 2: Relationship between FTIR parameters and age (A: carbonate: phosphate values vs age, B: Phosphate: amide values vs age), for fracture and non-fracture specimens. Errors have been excluded from the graphs for clarity.
Linear Regression Analysis | ANOVA
---|---
Non - Fracture Correlations with Age | Non - Fracture vs Fracture (Age Matched)

| Phosphate: | 0.023 | 0.13 | 0.05 ± 0.02 | 0.403 | Mean Difference (Non Fracture – Fracture) |
| Amide | | | | |
| Carbonate: | 0.988 | 0.00 | - | 0.013 | -0.002 ± 0.0005 |
| Phosphate | | | | |

Table 4: *Left:* p – values and $R^2$ calculated from linear regression statistical analysis when comparing the various FTIR material characteristic parameters and age for the non-fracture group. For the parameters were a significant trend was observed, the rate of change ($\Delta$) per 5 years is also reported. *Right:* 3: p – values for age matched ANOVA analysis of fracture (n = 15) and non-fracture groups (n= 22), for each FTIR material characteristic parameter. The mean difference between the non-fracture and fracture groups is also reported, for those parameters found to be significantly different (p > 0.05).

**Microarchitecture and Material Characteristics**

We have also examined correlations between the material properties and the architectural properties for the fracture group, in order to explore the potential influence bone chemistry may have on the architecture of compromised specimens. There is significant evidence from previous studies that bone mechanical properties (e.g. fragility) are affected by physicochemical material features [14, 38 - 42], although there remains controversy concerning the precise nature and magnitude of such relationships. The composition of apatite is known to markedly affect its crystallite size and shape. For example phosphate substitution by carbonate (bone apatite contains ~5 % wt CO$_3^{2-}$) results in smaller crystallites than the corresponding unsubstituted chemistry [43], as the increase in lattice disorder produces increased solubility of the crystallites [44].

**Coherence length**

$<00\ell>$ coherence length values were found to correlate with various microarchitecture parameters previously reported for the fracture group (Figure 3). With increasing coherence
length values, an increase in trabecular thickness (TbTh) ($p = 0.028$, $R^2 = 0.24$, Figure 3A), tissue mineral density (TMD) ($p = 0.006$, $R^2 = 0.35$, Figure 3B) and bone volume to total volume (BV/TV) ($p = 0.036$, $R^2 = 0.22$).

![Graph A](image1)

![Graph B](image2)

Figure 3: Relationship between microarchitecture properties and coherence length (00ℓ direction), for fracture specimens, A: TbTh vs CL, B: TMD vs CL. Errors have been excluded from the graphs for clarity.
Discussion

Although bone performs biologically critical mechanical and homeostatic functions [46], the relationships between its hierarchical constituents are not well understood. However, in contrast to previous studies where the osteoporotic stage of the bone is unknown (e.g. Ovx) [14, 22, 47], our specimens have fractured and therefore present particularly compromised material. Further the power of some previous studies [16, 19, 20, 28] would be insufficient to demonstrate the significant differences observed within our work. The basis chemical composition (non-stoichiometric hydroxyapatite), crystallises into a variable ultra-structure (nano-crystallites) that together with organic components form the fundamental building blocks of bones microarchitecture. Thus, in order to understand mechanical failures associated with compromised bone tissue such as osteoporotic material, it is crucial to understand the fundamental chemistry of the biological mineral. Current literature focuses on the micro architecture of bone [10 – 12], but few studies have investigated the material crystallographic characteristics of compromised bone mineral [13 – 18]. The potential influence of changes at the nano scale (i.e. in the mineral chemistry) on the micro architecture of human bone has, to the authors’ knowledge, not previously been directly considered. X-ray coherent scatter provides information specifically regarding the physicochemical characteristics of bone mineral.

Differences in the material properties between osteoporotic and ‘normal’ material, particularly derived from X-ray diffraction, are not consistent across the literature [13, 19, 20, 22, 28, 29]. The absolute values for coherence length from this study are consistent with previously published examinations of human bone [28, 48, 49]. However, the lattice parameters reported herein are significantly less than those reported previously [28, 29]. For this study, an internal reference in the form of silicon was employed to ensure accuracy and our values are consistent with levels of carbonate substitution known to occur in bone [30]. Precision was crucial in this study as differences between fracture and non-fracture material were anticipated to be subtle. As previous studies do not use internal references, this could explain the difference in absolute values.

We were unable to detect significant changes in <00l> coherence length or lattice parameters with age. A previous study of Handschin and Stern [50] reported an increase in crystal length and perfection up to the age of 25 years old and no significant changes until the age of 50 years old, were the average length decreased. The observed changes at 25 years and 50 years
old may not have been apparent in the data presented in this study due to study power. Handschin and Stern utilised a significantly greater number of specimens (n = 117), across a wider age range (0 – 90 years old). A significant increase in coherence length along the <0k0> direction, which suggests an increase in crystallite size and perfection, was observed with age in the study reported here.

When age matched, significant differences between fracture and non-fracture material were demonstrated. The <00ℓ> coherence length was found to be significantly lower for the fracture group than the non-fracture group (p = 0.036), which is consistent with previous studies [19, 20]. Our study therefore suggests a smaller coherence length corresponds to a material which is more susceptible to fracture, suggesting the mechanical strength would be less than that of non-fractured material. Previous studies have suggested that crystal size is related to mechanical strength [51 - 53], with studies reporting that increased bone mineral crystal size is associated with increased bone fragility [51]. However, after reporting a decrease in crystal thickness with age as crystal length increases, Boskey and Mendelson suggested from their preliminary data that mechanical strength is greater when the average crystallinity is greater [54]. However, Boskey also argued in favour of an optimal situation in which there is abroad distribution of crystal sizes [15] and Fonseca et al. reported that bone strength is favoured by greater mineral crystal size heterogeneity [55]. Further, Chachra et al. [56] reported that a reduction in crystallite size of bone mineral is associated with a decreased load accommodation and increased fracture risk. This is has also been observed in pathologies such as osteoporosis imperfecta [57].

The ‘a’ axis lattice parameter values, which are rarely reported in the literature for bone material, were found to be significantly lower (p = 0.001) for the fracture group in comparison to the non-fracture group. In contrast, Mkukuma et al reported no significant difference between osteoporotic material and ‘normal’ tissue when considering lattice parameters [28]. However, the relative low power (low sample numbers) may not have revealed the relatively modest differences (< 1%) between osteoporotic and ‘normal’ tissue. The stoichiometric ‘a’ axis lattice parameter value for hydroxyapatite is reported as 9.42 Å [29]. The data reported herein suggests non-fracture material is more stoichiometric than the fracture material, and therefore more chemically stable. Changes to lattice parameters values are caused by ionic exchanges and vacancies which induce strain into the lattice. This changes the characteristics of the apatite which are critical to crystallite size and dissolution rate [58]. Greater dissolution rates, for example, will be observed for mineral lattices which
are highly strained and therefore less stoichiometric. As osteoporosis is a condition associated with bone loss and perhaps greater turnover, the more soluble lattice structure is probably a more likely state. The reduced lattice parameters calculated for the fracture group is consistent with the carbonate to phosphate ratios calculated with FTIR, as many studies have shown with increasing B–type carbonate, the ‘a’ axis lattice parameter is reduced [30, 59]. No significant difference was observed for the ‘c’ axis lattice parameters between the two groups. Changes to the unit cell through ionic substitutions has previously been shown to have more of an effect on the ‘a’ axis in comparison to the ‘c’ axis [30], which is evident when considering the influence of carbonate substitute on the lattice parameters. LeGeros reported a systematic change in ‘a’ that was 2.5 times that of ‘c’ for an equivalent increase in amount of B-type carbonate substitution [30]. Therefore we propose that the sensitivity of our study is too low to detect corresponding significant changes to the ‘c’ axis.

The material characteristics obtained from FTIR demonstrate an increase in phosphate to amide ratio with increasing age. This may suggest an increase in the amount of phosphate and/or a decrease in collagen (measured through the amide vibrational peak). An increase in mineral content with age has previously been reported [15], as well as an increase in collagen maturity, resulting in a decrease in collagen content [60]. When age matched, the phosphate to amide ratio was not significantly different between the fracture and non-fracture group, although the values for the fracture group were lower. In contrast, previous studies have shown a decrease in the phosphate to amide ratio in both ovariectomised monkeys and osteoporotic human tissue [14, 25, 34, 61], suggesting either a lower mineral content and/or a greater collagen content. The consensus tends to be that in osteoporotic bone, a decrease in mineral content is observed [14, 15], resulting in a reduction in mechanical strength [55].

Although carbonate incorporation has been reported to increase [50, 62, 63] and decrease [34, 64] with age, no significant correlation with age was observed for the carbonate to phosphate ratio values in this study. This is consistent with [52], who found carbonate incorporation remained constant after 45 years old. When age matched, a significant difference between fracture and non-fracture material was observed for the carbonate to phosphate ratios, with a mean difference of 0.002 ± 0.0004. The average value for the fracture group was significantly greater in comparison to the non-fracture group. This suggests greater carbonate content in the fracture material in comparison to the non-fracture material and/or less phosphate. A higher carbonate to phosphate ratio in the fracture material is consistent with previous studies [14, 23, 27], with a study by Boskey suggesting a 8% increase using FTIR [25]. This increase
is consistent with the data reported here, where a ~ 11% increase in the carbonate to phosphate ratio was observed for the fracture material.

In general, it is considered that newer bone contains a large quantity of carbonate and is characterised by smaller crystals [31, 65]. In an accelerated turnover system, the consumption rate of the more mature tissue (i.e. that preferentially targeted for remodelling) is enhanced [66]. Crystallite populations then become biased towards reduced average dimensions, larger specific surface areas and greater amounts of carbonate. The data presented here is consistent with this model, as the coherence length values are significantly lower for the fracture material whilst the carbonate to phosphate ratio was significantly greater.

This study has also examined relationships between the material characteristics of bone and its corresponding architectural properties, as there is significant evidence from previous studies that bone mechanical properties (e.g. fragility) are affected by physicochemical material features [14, 38 – 42, 51 – 54]. This is the first time these parameters have been compared and the study provides a materials science insight into how bone physicochemistry at the nanoscale potentially influences the architecture at the microscale. The results suggest that material characteristics, in particular the coherence length, potentially have an influence on various micro-architecture properties. Coherence length values are a quantification of crystallite disorder (size and microstrain). An increase in microstrain can indicate ionic substitutions and vacancies. This change can also have an effect on crystallite dimensions and other factors including solubility. Previous studies have shown the solubility of apatite increases as the lattice microstrain is increased [58]. Further, disorder within the apatite lattice, has been proposed as a fundamental contributor to bone mechanical compromise for more than three decades [51] and evidence for this hypothesis continues to be reported [20]. In this study, with increasing coherence length values, increases in TbTh, TMD and BV/TV were observed. It is proposed that bone apatite with higher coherence length values represents a more stable, less soluble material and therefore should be associated with increased amounts of bone mineral as reflected by the increase BV/TV, BMD and TbTh.

A limited number of fracture and non-fracture material from age matched male donors (7) were also examined. It was found that the phosphate to mineral ratio values were significantly greater for the fracture group, suggesting a reduction in collagen and/ or an increase in phosphate. In contrast, the carbonate to phosphate ratio values between the two groups was not significantly different. The tissue mineral density (TMD), was also
significantly different between fracture material collected from female and male donors; TMD was greater for the male specimens. This difference may be due to fundamental compositional differences between the female and male fracture material. For example, if the carbonate content is significantly greater in the female fracture group then this would reduce the apatite density (therefore TMD). Stoichiometric hydroxyapatite, ~3.2 g cm$^{-3}$ [67], has been shown to decrease with the incorporation of increasing amounts of carbonate [68, 69]. Reducing the amount of carbonate by ~50% produces an increase in density by ~15%.

Further, the phosphate to amide ratio was significantly greater for the male fracture material suggesting an increase in phosphate and/or a decrease in collagen, both of which would modify TMD values. An increase in phosphate would result in a more stoichiometric mineral, resulting in an increase in density, whilst a decrease in collagen, would result in an average increase in TMD. The differences in the fracture group between males and females, as previously hypothesised [70], maybe due to remodelling differences caused by different hormonal changes in men and women.

Conclusion

The principal main aim of this study was to investigate bone ‘quality’ in terms of mineral chemistry and organic content of fractured and non-fractured human material. Age related changes for the non-fracture material were also investigated. For the first time, the relationship between the material characteristics and the micro architecture parameters was examined. As shown through statistical analysis, the coherence length ($<00\ell>$), ‘a’ axis lattice parameters and carbonate to phosphate ratio values (when age matched) were significantly different between fracture and non-fracture material from female donors. The study has also shown an increase in crystallite size and perfection ([0k0]) as well as an increase in the amount of phosphate and/or a decrease in collagen occurs with age for non-fracture material. It is proposed the data reported here suggests osteoporosis may not simply be an accelerated aging process when considering mineral chemistry and organic content, but there are fundamental chemical differences between fracture and non-fracture material not found with age. It is interesting to speculate that the mineral properties of bone at the ultrastructure level may influence the micro architectural properties of trabecular bone. A preliminary investigation into the material properties of fractured and non-fractured material from male donors, although not presented here, suggested differences in the material quality between fracture males and females. It is proposed these differences translate through to
measured TMD values and potentially highlight differences in bone remodelling between fracture males and females.

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References


