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Towards new material biomarkers for fracture risk

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ABSTRACT

Osteoporosis is a prevalent bone condition, characterised by low bone mass 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 physicochemistry 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 trabecular bone at both ultrastructure and microarchitectural levels to provide a detailed material view of bone, and therefore provides a more comprehensive explanation of osteoporotic fracture risk. Physicochemical characterisation obtained through X-ray diffraction and infrared analysis indicated significant differences in apatite crystal chemistry and nanostructure between fracture and non-fracture groups. Further, this study, through considering the potential correlations between the chemical biomarkers and microarchitectural properties of trabecular bone, has investigated the relationship between bone mechanical properties (e.g. fragility) and physicochemical material features.

1. 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 DEXA is not without limitations and is arguably 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 limitation of DEXA was perhaps highlighted with the development of FRAX, the World Health Organisation (WHO) fracture assessment tool, which uses BMD along with clinical risk factors and country-specific fracture and mortality data to quantify a patient's 10-year probability of a hip or major osteoporotic fracture [9]. FRAX takes into account demographic information such as age, sex, a prior fracture, family history of fracture, and lifestyles risk factors such as physical inactivity and smoking.

Arguably, the limits associated with DEXA to predict an individual patient's fracture risk is because BMD does not measure the multiple material factors that contribute to bone strength [10]. There are several complex determinants of bone strength and fragility, and although properties which may increase the resistance to one type of mechanical demand, for example static loading, may also be detrimental to other kinds such as fatigue loading [11]. For this reason, material scientists differentiate between stiffness: how well a material resists deformation, toughness: the ability of a material to absorb energy prior to failure and strength: the ability of a material to resist failure when stretched or compressed. In this manuscript, strength refers to the ability of the material to withstand loading before structural failure occurs. The

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assumption is that bone strength is lower for the fracture group than that of the non-fracture group. Further mechanical testing would be required to establish whether bone chemistry parameters investigated in this study are independently correlated to stiffness, toughness or strength.

In this context, 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 [10]. 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 (due to osteoporosis or osteoarthritis) when compared to healthy controls [12–14]. This has recently led to the investigation of various imaging techniques including high-resolution peripheral quantitative computed tomography (HR-pQCT) and the development of analytical tools such as trabecular bone score (TBS). Unfortunately, HR-pQCT is restricted to peripheral skeletal sites and therefore the lumbar spine or proximal femur (common sites for osteoporotic fragility fractures which are associated with the most significant quality of life burden for patients) cannot be imaged with this technique [15]. TBS, which captures information relating to trabecular microarchitecture by performing novel grey-level texture measurements on DEXA images [16], is undoubtedly promising; however, this tool quantifies the microarchitecture of the bone only and does not account for bone chemistry.

Bone chemistry is more complex, with studies often providing contradicting results and conclusions [17–23]. Unfortunately, many of the studies which investigate the chemistry of osteoporotic bone are limited by relatively low sample numbers ($n \leq 10$ for both osteoporotic and 'normal' specimens) [20,24,25] and/or utilise ovariectomized (Ovx) animal models [26,27]. Exceptionally, a more recent study by Boskey [22], investigated the material properties of a large number of cortical and trabecular specimens ($n = 120$) using Fourier transform infrared spectroscopy (FTIR). 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. Several previous studies have examined the physicochemical properties of the inorganic bone component (i.e. the hydroxyapatite mineral) characterised by X-ray diffraction (XRD) [17,24,25] and the organic component (i.e. collagen) as characterised by Raman spectroscopy [28,29] or Fourier transform infra-red spectroscopy (FTIR) [26,30–33]. Perhaps of most relevance are those reports detailing differences between osteoporotic and 'normal' tissues, although inconsistencies are frequent. For example, reports such as those of Thompson et al. [17] and Faibish and Boskey [34] suggested an increase in crystallite size in osteoporotic tissue. However, these two reports differ in conclusions regarding the crystal chemistry; Thompson suggested a decrease in carbonate [17], whilst Faibish and Boskey [34] argued for an increase when comparing osteoporotic to normal bone. An increase in both crystallite size and carbonate content was reported by Gadeleta et al. [18]. Several reports have been unable to demonstrate significant differences between osteoporotic and normal bone tissue when considering crystallite size [20,27,35] although a review by Boskey in 2003 reported that the general consensus accepts that osteoporotic bone mineral has larger crystallites than the non-osteoporotic counterparts [19]. It is evident from the literature this viewpoint is contentious. A more recent study by Boskey et al. [22] reported a decrease in carbonate to phosphate ratios in fractured bone compared to non-fractured cortical bone, suggesting either a decrease in carbonate and/or an increase in phosphate. No other 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 [28].

There have been a few studies that have examined changes to the hydroxyapatite unit cell parameters (as a proxy for lattice substitutional modifications) of osteoporotic and/or aged bone mineral [35,36]. 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) [37,38]. In general, a decrease in 'a' axis and an increase in the 'c' axis lattice parameters have been reported with age [36]. These trends are consistent with increased in B-type carbonate substitution [37] observed in synthetic apatites. In contrast other studies were unable to detect differences in the lattice parameters of osteoporotic bone [35]. As a further bone characteristic measured by FTIR, it has been reported that for osteoporotic tissues the mineral to organic ratio is lower than that of normal bone [18,30]. Thus in general, the literature contains several previous studies of bone physicochemical characteristics but these very often provide apparently conflicting findings and results. This, in the context of our work, is considered in further detail within our Discussion section.

The study described herein 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 the potential relationship between the bone mineral chemical properties – and the microarchitectural properties of bone was investigated. This novel component of the work only involved the fracture group as relatively large deviations in architecture would be expected in this group.

2. Materials and methods

2.1. Bone specimens

A sample set of 20 femoral heads were collected from osteoporotic female patients who had suffered fragility 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, ranging from 59 to 91 years old. 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.

2.2. Sample preparation

Trabecular bone was obtained and analysed for this study from the femoral head. Overall the strategy was to select random samples with respect to femoral head location although each sample was cut to include tissue from at least two quadrants of the head. The femoral head is often defined into four quadrants: anterior, posterior, inferior and superior [39,40]. It is important to note that samples were obtained from trabecular bone only, and did not include cortical bone. For a complete description of the sampling and sectioning procedure refer to [41–43]. 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 1 min. Once powdered, the specimens were sieved through a stainless steel mesh sieve of 106 μm to ensure a homogenous fine powder sample.

2.3. X-ray diffraction (XRD)

The powdered trabecular bone specimens were individually loaded on to low background scattering (off-cut silicon) XRD holders. The bone powder was spiked with a NIST standard reference silicon powder