Chapter 7

Summary of conclusions and indications for further work

7.1 Overview of aims and summary of conclusions

Osteoarthritis is by far the commonest musculoskeletal disability in the developed world and represents a significant health burden to modern society. In the UK, it is estimated that 34 % of the population over 45 yrs have OA at the knee, and 19 % of those over 55 yrs have OA of the hip. Interestingly, osteo-archaeological evidence indicates that OA has affected human populations for millennia [Larsen 1999].

The hip joint experiences some of the greatest and most complex mechanical loads the body has to withstand. This is reflected by the great degree of anisotropy of the cancellous bone within the femoral head. In particular, two regions are readily identified. The superior region which is a major force-bearing zone, and below it the inferior region, a site which in normal circumstances bears less forces than the superior region, probably as a result of stress shielding. The cancellous bone comprising both regions is expected to remodel through adaptive processes largely governed by the forces experienced by each region. It thus follows that bone turnover in these regions will differ and that this may be reflected in some way by alterations in the composition of the bone matrix.

Osteoarthritis is a condition which eventually affects all the tissue compartments of the joint. On a macroscopic scale, bony changes including sclerosis, growth of osteophytes, alterations in femoral head shape and in some cases increased bone density have been described [Knight *et al.* 1992]. Moreover, a common clinical observation is that patients with OA are not likely to develop osteoporotic fractures [Dequeker & Johnell 1993]. This led to speculation that bone affected by OA is stronger than that affected by osteoporosis. It follows that end-stage OA is likely to be a suitable pathology to investigate mechanical and compositional relationships in cancellous bone.

Of the few detailed chemical analyses of OA bone to have been reported the majority have highlighted alterations in the underlying bone which include:

- An undermineralized bone matrix [Grynpas *et al.* 1991; Li & Aspden 1997; Mansell & Bailey 1998].
- Increased bone turnover [Reimann & Christensen 1979; Mansell *et al.* 1997; Mansell & Bailey 1998].

- Changes to the structure of the trabecular network [Fazzalari & Parkinson 1997], and more recently
- The presence of the type I collagen homotrimer variant [Bailey *et al.* 2002].

The aims of this thesis were:

1. **To characterize some of the physical and compositional properties of cancellous bone from the Superior and Inferior regions (Sup and Inf) of Normal and OA FHs including:**

- Material properties - ρ_A and ρ_T,
- Mechanical properties - E_C, σ_Y and W_Y,
- Matrix components
  - mineral and collagen content,
  - regulatory proteins (osteocalcin and insulin-like growth factors),
  - crosslinked telopeptide domains of the structural collagens type I and III (ICTP and IIINTP).

2. **To compare and contrast these properties between:**

- Cancellous bone cores taken from the Sup and Inf regions of the femoral head,
- Normal and OA groups.

3. **To investigate effects of the compositional parameters on the material and mechanical properties.**

From the work of others it was predicted that

- *OA bone would have an increased ρ_A, and since mechanical properties have a high dependency on ρ_A, this would be reflected by an increase in the mechanical parameters, such as, stiffness.*

The findings reported here suggest that in both Sup and Inf regions of the OA FHs, ρ_A was increased, but only significantly at the Inf site (Table 4.6). However,

none of the mechanical properties were significantly increased in the OA bones studied (Table 4.7).

- *OA bone matrix would be undermineralized.*

This study found OA bone to have a significantly reduced mineral:collagen ratio in the Sup region, but this was not evident within the Inf region (Table 5.1).

- *Markers of osteoblast activity, such as OC, would be increased in OA.*

Matrix OC concentrations in OA bone were not significantly different to Normal (Table 5.6).

- *Regulatory proteins, such as, IGF-I and -II would be increased in OA.*

No significant differences were found between either IGF-I or -II in either the Normal or OA bones (Table 5.7).

- *Increased matrix turnover in OA would be reflected by a reduction in the trivalently crosslinked ICTP structures of type I collagen.*

The converse was found. ICTP was significantly increased in the mineralized collagen matrices of both the Sup and Inf regions of OA bone (Table 5.2). This suggests several possible explanations:

(i) Increased lysyl hydroxylation at the C_t telopeptide and an increase in trivalent crosslink formation,

(ii) The presence of, or an increase in type I collagen homotrimer. The additional $\alpha 1$(I) chain results in an extra C_t telopeptide Lys residue available for collagen crosslinking.

Additionally, it was possible to predict certain differences between the Sup and Inf regions of both Normal and OA FHs, these included:

- *Increased ρ_A within the Sup region relative to the Inf site and a concomitant increase in E_C, σ_Y and W_Y.*

In both Normal and OA groups, ρ_A was significantly increased in the Sup region with this increase being reflected by the mechanical properties (Tables 4.6 & 4.7).

This study produced several unexpected, novel findings:

- Altered relationship between ρ_A and E_C in the OA group (Figure 4.3B) resulting in a tendency for OA bones to have a lower E_C value for the same ρ_A measure as those from the Normal group.
- Increased type III collagen within the mineralized matrix of OA bone (Table 5.2).
- Increased IGFBP-5 within the OA bone matrix (Table 5.7).
- Loss of negative associations between both IGFBP-5 and OC with ρ_A in the OA group (Figures 6.2 & 6.3 respectively).

7.2 Limitations of the current study and possible improvements

In this pilot study several limitations were identified. These are discussed below, along with suggested improvements:

- *Collection of bone specimens.*

By using specimens collected from subjects of the same gender and from a narrower age range, variation in these parameters would have been minimized. Furthermore, by increasing the number of subjects within both groups, the biological and statistical significance of the data would have been improved. This study was limited to FHs made available from post-surgery and post-mortem. With the data collected in this work, it will now be possible to perform power calculations to design future studies examining some of these parameters in bone. These future studies could look at other common bone pathologies such as osteoporosis, rheumatoid arthritis and Paget's disease of bone.

- *A sample site where the cancellous bone architecture is more isotropic.*

Choice of a skeletal site that has a less complex cancellous bone structure, which is more isotropic, than the sites studied at the femoral head, and is readily

available from surgery and at autopsy. For example the tibial metaphyses, from which bone is often available after total knee replacement.

- *To make direct measurements of architectural parameters.*

A number of parameters have been defined so that the trabecular architecture of cancellous bone can be quantified [Odgaard 1997]. The simplest technique for evaluation of these is histomorphometry. Alternatively, techniques such as micro-computerized tomography (μ-QCT) or high-resolution nuclear magnetic resonance (NMR) imaging enable the production of a digitized image from which computer algorithms may rapidly generate values for the architectural parameters.

- *Mechanical testing of individual trabeculae.*

Measurements of the elastic modulus of individual trabeculae are now readily performed using a microtensile technique described by Rho *et al.* [1993]. Changes in the mechanical properties of the individual trabeculae would be more directly related to alterations in composition rather than influenced by architectural parameters.

- *Direct measurement of the displacement occurring within the specimen during compression testing.*

This would be possible by obtaining and attaching displacement transducers directly to the bone specimens. This would ensure that the displacement measured does not include any displacement occurring within the compression cage.

- *Direct measurement of collagen crosslinks.*

This would have supplemented the ICTP data and provided indications of which of the known collagen crosslinks are increased within the mineralized collagen matrix of OA bone.

Additionally other methodologies providing an indication of the extent of tissue turnover would add further depth to the current study and include:

- The use of back scattered electron-scanning electron microscopy (BSE-SEM) [Jones & Boyde 1994] which provides information relating to temporal and pathological changes in the composition of the mineral phase of bone.

- Combined use of the α- and β-specific forms of the CrossLaps assay to detect the degree of β-isomerization in the α1(I) C_t telopeptide [Fledelius *et al.* 1997b]. β-isomerization is the consequence of a non-enzymatic, physiological ageing reaction. An increase in β-isomerization indicates that the collagen peptides are older (more mature) than peptides containing α-form.

By taking into consideration the findings and the possible improvements to this study, it is considered relevant for similar investigations into other bone pathologies such as:

- Paget's disease of bone,
- Rheumatoid arthritis and
- Osteoporosis

The mechanical competence of the cancellous bone may be influenced by changes in composition in these pathologies. Furthermore, the identification of compositional changes and quality of OA bone may be a factor worthy of consideration prior to its use by orthopaedic surgeons in bone grafting. This study has highlighted that the stiffness of OA bone tends to be lower for the same ρ_A than Normal bone, and therefore may impair the success of the bone graft.

7.3 Further work

Future laboratory investigations could investigate:

- Control and regulation of bone cells and how they communicate with one another.

- Measurement of other IGF system components that may influence the concentrations of those IGF system components described here such as IGFBP-3 and -4.
- Identification of patterns of mRNA expression of the various IGF system components in healthy ageing and OA.

Future *in vivo* work could investigate:

- Direct measurement of type III collagen and IGFBP-5 in bone biopsies. This approach may identify early OA changes within the bone matrix prior to those changes affecting the articular cartilage and other tissues of the joint.
- Comparison of serum IGFBP-5 concentrations in health and OA.
- Measurement of specific markers of bone turnover within the synovial fluid, blood or urine including bone alkaline phosphatase (BALP) isoforms, osteocalcin, PINP and PIIINP and other metabolites of collagen.
- Anthromorphometric comparisons between those patients with OA and those with other musculoskeletal disorders.
- Genetic studies to investigate markers for OA susceptibility. Recent published examples of this include polymorphisms in the Sp1 binding region of the COLIA1 gene [Mann *et al.* 2001] and in the TSG-6 gene that codes for a 35 KDa protein that binds the cartilage matrix [Nentwich *et al.* 2002].

In conclusion, femoral head cancellous bone in end-stage osteoarthritis is different to that from healthy subjects. The cancellous bone has a greater apparent density (ρ_A), but this is not reflected by the mechanical properties. The relationship between stiffness and apparent density is altered, with a tendency for E_C values to be lower for the same ρ_A than in healthy bone. Furthermore, OA bone is undermineralized; this is especially evident in the superior region. In the OA mineralized matrix (ICTP) was increased, possibly reflecting the undermineralized nature of the bone matrix. Type III collagen, determined by the presence of IIINTP and PIIINP antigens, was increased suggesting either an altered osteoblast phenotype or deposition of fibrous granulation tissue in response to fatigue. Although the anabolic growth factors, IGF-I and IGF-II were not altered, OA bone matrix

contained increased concentrations of the binding protein IGFBP-5. This factor may be responsible for maintaining bone mass in OA. IGFBP-5 and the matrix binding protein osteocalcin correlated inversely with ρ_A in healthy bone, but these associations were lost in OA indicating a loss of regulation at this level in OA.