3 BONE GRAFT PREPARATION

3.1 Human and xenograft bone

- Human bone
Morsellised bone grafts were produced from human femoral heads as the gold standard in clinical impaction grafting and from the bone of ovine humeral and femoral heads which were tested and used as experimental xenograft for the majority of the tests. Human femoral heads were donated from stocks of the Department of Orthopaedic Surgery, University of Bristol. After femoral heads had been harvested from the donor they were either frozen at -80°C from fresh or first irradiated at two different irradiation levels.

![Figure 3.1: Human femoral heads prior to milling.](image)

Figure 3.1 shows the large variation found in human donor femoral heads. Before the preparation of morsellised bone chips the femoral heads were thoroughly defrosted at room temperature for a minimum of 3h. Then soft tissue such as residual ligaments, fat and cartilage were removed with a scalpel as illustrated in figure 3.2. Large femoral heads were sectioned into two to four parts to make them fit down the feeder tube of the bone mill. The bone was then milled using either a Norwich or Howex bone mill. Cortical bone fragments or bits of soft tissue easily identifiable to the eye were manually removed. Bone fragments which came out too large when the Norwich mill was used, were cut down to size with a pair of cutters. The morsellised graft was mixed to level out the variability in bone quality from different donors. Fresh, in other words un-treated, bone and irradiated bone were then used for experimentation. A part of the human graft was fixed in a 10% formalin solution for 48h or washed and towel dried. As not all graft prepared could be tested on the same day, residual quantities were stored at -30°C and thoroughly defrosted again at room temperature prior to
re-use. Due to this procedural constraint, the influence of multiple freeze-thaw cycles on the mechanical properties of the graft were investigated as well (see section 4.4.1). The preparation steps of morsellised human bone graft can be summarised as follows:

- Defrosting femoral heads for a minimum period of 3h at room temperature
- Removal of visible soft tissue with scalpel
- Sectioning femoral heads with hacksaw to fit feeder tube
- Milling with Norwich or Howex bone mill
- Experimental use or fixation with formalin or washing & drying
- Residual grafts are stored in a freezer at -30°C
- Prior to re-use: Defrosting frozen grafts for a minimum period of 3h at room temperature

Figure 3.2:
Removal of soft tissue like cartilage from the human femoral heads.

- **Ovine bone**

Ovine morsellised bone graft as the major experimental xenograft used in this study, was prepared in a similar way to human graft. Ovine humeral heads were donated by a local butcher which ensured a bone supply with relatively constant properties regarding size and age of the sheep. The ovine humeral heads were stored in a freezer at -30°C and were thoroughly defrosted for a minimum period of 3h prior to preparation. At first large quantities of soft tissue like muscles, flesh, tendons, ligaments and cartilage left over from butchering had to be removed. Then the humeral head was cut off from the proximal femur just below the cartilage line at the distal end of the trochanter using a hacksaw. Figure 3.3 shows the results of these preparatory steps. The humeral heads were then milled using the Norwich bone mill with the coarse blade. The morsellised graft of 10 to 20 humeral heads was prepared and mixed at once to level out variability. The bone chips were then fixed in a 10% formalin solution for 48h after which the graft was washed with tap water through a 250μm sieve and subsequently towel and air dried for 1h to a constant level of residual wetness
comparable to that of the fresh bone. In between test sessions remaining bone graft was again stored in a freezer. The preparation steps of morsellised ovine bone graft can be summarised as follows:

- Defrosting ovine femur for a minimum period of 3h at room temperature
- Removal of soft tissue with scalpel
- Sectioning of humeral head below cartilage line with hacksaw
- Morsellising bone with Norwich® bone mill (coarse blade)
- Fixation in formalin for 48h and subsequent washing & drying
- Residual grafts are stored in a freezer at -30°C
- Prior to re-use: Defrosting frozen grafts for a minimum period of 3h at room temperature

![Figure 3.3: Ovine humeral heads prior to milling.](image)

A major difference between the human and ovine bone is the size of the femoral or humeral heads and the ratio between endosteal cancellous bone, periosteal cortical bone and other tissue. However, human donor graft is usually of weak trabecular integrity. As a result, despite the size advantage, graft quantities that can be harvested from one human femoral head and one ovine humeral head were similar with 36.2g human graft/head versus 39.9g ovine graft/head. Another visible difference between freshly morsellised human and ovine graft is the higher blood content of the human bone as represented in figure 3.4. After slaughtering sheep are drained of blood which is further reduced by the meat production process whereas the human femoral head is stored immediately after osteotomy so that no blood is lost. However washing and drying the human and ovine graft produces bone chips of visually similar appearance hinting at a comparable particle size distribution and cancellous bone to soft tissue ratio as can be seen in figure 3.4. When comparing the wet with the washed and dried bone, in particular with the human bone, it also becomes evident that fat and blood
bind small graft particles into agglomerations of bone chips. The washed and dried human bone appears entirely different in particle size distribution to the wet bone. How human, ovine and bovine bone graft compare mechanically was studied in chapter 4.4.1.

![Figure 3.4: Comparison of human and ovine bone graft morsellised with a Norwich bone mill. Grafts are compared fresh from mill and washed and dried.](image)

- **Bovine bone**

Bovine bone was also investigated as a potential xenograft for experimental purposes. The large size of its femoral and humeral heads promised efficient graft harvesting and a reliable source of a xenograft bovine bone chips for *in-vitro* testing. However concerns about BSE limit availability and the legal laboratory use of bovine graft. Bovine graft tested in this study for comparative purposes was imported to Britain and was only used in a biohazard laboratory. During the preparation of bovine bone graft it became obvious that, in addition to these disadvantages, bovine bone might not be suited as an experimental graft because the density of its trabecular structure is much higher than both human and ovine bone (see figure 3.5). Bovine femoral and humeral heads cannot be morsellised with the Norwich mill. Only
the Howex bone mill with its strong and protruding blades could be used successfully. The cancellous bone is very dense and increases from the centre of the heads towards the outer surface such that the cancellous endosteal and the cortical perioskeletal bone cannot be distinguished. The same phenomenon and density was found in equine bone also investigated briefly as a potential source of an experimental xenograft.

![Sections of bovine femoral head](image)

**Figure 3.5**: Sectioned bovine humeral heads prior to milling (left). Comparison of human and bovine graft morsellised with the coarse and fine blades of the Howex mill (right).

### 3.2 Ceramic bone graft extenders

The synthetic bone graft substitutes investigated were ceramic composites of hydroxyapatite and tri-calciumphosphate in different configurations. The ceramic material was produced by TCM Associates, Neizing, UK, a medical device manufacturer sub-contracted by StrykerHowmedicaOsteonics. In order to protect intellectual property rights, the production process is not fully disclosed but relevant information can be given.

Hydroxyapatite and tri-calciumphosphate powders plus an organic material for pore creation are mixed as desired. The weight ratio of the hydroxyapatite (HA) and tri-calciumphosphate (TCP) powders defines the chemical composition produced and tested, such as 80:20, 50:50, 20:80 HA/TCP. The powder is cold-pressed into cylindrical tablets of ca. 50mm diameter and 10mm ca. height. The tablets offer sufficient integrity to be handled and be put into a device which crushes the tablets into granular fragments by pushing a stamp with pins geometrically
arranged in a chess board pattern through the tablet. Depending on whether large or small particles are desired, the process is repeated with the fragmented tablet as often as required. The particles are manually sieved to remove dust and create the desired size interval. The remaining granules are then sintered at a temperature range from 1050°C to 1200°C with a preset and constant heating and cooling rate. Porosity was created by an organic additive material which acts as a porosifier by burning off, free of residuals, during sintering.

Porosity levels of the ceramic bone substitutes are given in percentage values and are derived from the weight percentage of the organic agent added to the ceramic powder mix. Thus they represent relative porosity levels with reference to the manufacturing extremes "no porosity" (0%), "medium" (25%) or "high porosity" (50%) and do not give the exact ratio of void space. Exact porosity measurements using weight and volume differences between porous and bulk material or image analysis of cross sectioned samples have not been made. Porosity measurements of individual granules did not seem appropriate because the particle size was too small relative to the pore size and with an inhomogeneous porosity distribution concentrated in the regions of the outer surface a single porosity percentage would not describe porosity in a meaningful way.

The ceramic granule sizes tested have been chosen according to the typical particle size interval produced when milling human bone. The size ranges were small (1-2mm), medium (2-4mm) and large (4-6.2mm) and were prepared by sieving with British Standard sieves and an Octagon® shaker. A single charge of 40g ceramic was sieved for two minutes at intensity level 6. The property range of ceramic bone substitutes investigated was:

Sintering temperature: \( T_{\text{sim}} = 1050°C, 1150°C, 1200°C \)
Porosity levels: Zero (0%), medium (25%), high porosity (50%)
Size range: small (1-2mm) medium (2-4mm), large (4-6.3mm)

A 80:20 HA/TCP ceramic sintered at 1150°C with 25% porosity and a medium 2-4mm particle size was chosen to be the standard property configuration. A high hydroxyapatite ratio was seen as a most likely compromise for clinical application balancing the higher strength and more bone like mineral composition of HA with the higher dissolution rate of TCP. For sintering temperature, porosity and size interval, the median values of a sensibly chosen wide property range were selected as the standard. During testing only one of those parameters was changed at a time. Figure 3.6 shows a 50cm³ charge of the standard ceramic,
the 80:20 HA/TCP composite sintered at 1150°C with 25% porosity and a 2-4mm medium particle size. Figure 3.7 represents the particle size ranges tested.

**Figure 3.6:** 50cm³ charge of the experimental ceramic bone graft substitutes in standard property configuration: 80:20 HA/TCP, T_{sint} = 1150°C, 2-4mm.

Sintering a biphasic HA/TCP without any organic agent for pore creation results in a virtually non-porous material with few non-interconnected small voids on the surface measuring approximately 1-4μm in diameter. In order to be osteoconductively active, pores have to measure between 200 and 500μm in diameter and be interconnected. Figure 3.8 shows two images at different magnification of such a non-porous biphasic HA/TCP as used in the experiments.

**Figure 3.7:** Experimental ceramic bone graft substitutes (80:20 HA/TCP, T_{sint} = 1150°C, 25% porosity) sieved to three different size intervals.

In addition to the HA/TCP composite graft extenders described above, the prototype of another ceramic bone substitute from an alternative manufacturer was also tested for comparison. The pure HA granules offered an interconnected porosity of 70% and were available in a size range from 2-5mm.
3.3 Mixes of bone and ceramic graft extenders

In addition to bone grafts and ceramics, different graft mixes of bone and substitutes were investigated. While the ceramic properties were varied according to the list above, only fixed ovine bone was used to prepare different bone/ceramic mixing ratios. From clinical observations of the processing and handling issues during impaction grafting operations it was decided to prepare graft mixes by volume and not by weight. Although bone graft and ceramic substitutes have very different densities and although weighing small quantities of compressible granular material is more accurate than measuring volumes it was seen as a more practical and efficient approach. Plastic containers of the most commonly used volumes (25cm$^3$ to 50cm$^3$) were cut to size and graft samples were prepared by filling the appropriate container until a pile has formed which was levelled off using a spoon. During the filling process the container was tapped lightly by hand to allow some settling and redistribution of the particles. Despite the concerns about charging accuracy with this volumetric measurement, the average weight prepared with a container of one volume varied by less than 5%. Bone and ceramic were mixed in the ratios 2:1 bone/ceramic (b/c), 1:1 b/c and 1:2 b/c. Considering the different densities of bone and ceramic this converts to equivalent weight ratios of approximately 4:3 b/c, 2:3 b/c, 1:3 b/c (ceramic density varies especially with porosity). Figure 3.9 shows an example of fixed ovine bone and the standard HA/TCP ceramic (80:20 HA/TCP, $T_{sint}=1150^\circ$C, 25% porosity, medium 2-4mm particle size) in 1:1 bone/ceramic mix before and after mixing. Comparison is made to 2:1 b/c and a 1:2 b/c mix after blending.
Figure 3.9: Different volumetric mixes between ovine bone and the standard ceramic.

The delivery of a bone/ceramic mix sample for a test procedure required measuring a sample volume as described above but with special attention paid to conserving the original mixing ratio and achieving a homogeneous distribution across the container. Due to the different densities of bone and ceramic, the adhesive attraction of fatty bone and the wide size range of the bone chips (ca. 0.1 to 10mm), handling the graft easily leads to segregation of, for instance, large ceramic and large bone particles and undesired concentrations of, for instance, small ceramic particles. The same problem had to be considered during charging the measured volume into the experimental rig. For example emptying the container at a slow rate also resulted in segregation of the graft phase.