Allograft Bone and Super Critical Fluid (SCF) Technology

Summary:

1. Supercritical Fluids (SCF) have the properties of a gas and a liquid when it is in its supercritical state;
2. SCF treatment provides a delipidiation stage to ensure clean allograft tissue;
3. The current Therapeutic Goods Administration code of Good Manufacturing Practice – Human Blood and Tissue defines sterile as “free from viable micro-organisms”. Supercritical Fluid Technology in use within our facility has been proven to render all treated allografts sterile therefore allowing Australian Biotechnologies to label all treated Allografts with the sterile label.

Allografts

Allograft tissues provide surgeons with a reliable and convenient source of human tissue for use in reconstructive surgery of bone defects. The goal of using bone allografts is to initiate a biological healing response from the host bone bed and promote new bone formation at the surgical site. In contrast to autograft, the use of allograft bone is associated with certain advantages. Harvest of autograft is limited and an additional surgical procedure is required which lengthens the surgical time, increased blood loss, and results in persistent donor site morbidity, residual pain and cosmetic disadvantages (Arrington et al., 1996, Banwart et al., 1995, Goulet et al., 1997, Younger and Chapman, 1989). These complications are averted with the use of allograft bone.

Allograft bone, although at a very low risk, may transmit infectious diseases, thus safety is of prime consideration. In order to increase bone allografts biological potential and decrease the risk of infectious diseases, all bone allograft is required to undergo specific processing techniques before ultimately being terminally sterilized. The success and efficacy of these processing techniques are measured by the ability to preserve the mechanical and biological properties of the allograft while inactivating any potential infectious diseases and bacterial contamination.

Processing

Bone processing relates to all actions taken on donated bone tissue. Bone is retrieved, shaped and sized under aseptic condition. It is cleaned of any antigenic materials such as: bone marrow, lipids, and cellular debris. Finally it is terminally sterilized. The success of the cleaning processes is considered to be the most important step in bone allograft processing and ultimately dictates the osteoconductive capacity of the allograft in vivo.

To clean bone and remove unwanted materials various
fluids, detergents and selective extraction solvents are all commonly used. Furthermore, considering the microporous nature of bone it is of utmost importance for the cleaning fluids to penetrate the solid microporous structures which are incased by the bony tissue.

Super Critical Fluid

Carbon Dioxide (CO2), under the right conditions, namely, high pressure and constant temperature, reaches a state called supercritical. In this state, above its critical temperature and pressure (Tc = 31.1°C and Pc = 73.8 bar respectively) CO2 becomes what is called a Super Critical Fluid (SCF) and it is defined as a substance that exists in two distinct states i.e. liquid and gas. In this state, Super Critical CO2 (SCCO2) is considered one of nature’s best and most benign solvents as it exhibits many favorable properties, such as liquid-like density, high diffusion coefficient, gas-like viscosity, and gas-like surface tension that approaches zero (Fages et al., 1994a). The low viscosity means it flows unusually well with low resistance, and the almost-zero surface tension means the fluid’s surface doesn’t curl up at the edges nor stick to the sides of the material, ultimately increasing SCF’s penetrability and its dissolving power; enabling it to diffuse through the microporous structure of bone matrix to efficiently and effectively remove any unwanted antigenic materials. Thus, Super Critical CO2 technology has the potential to increase the overall permeability and surfaces area of bone by allowing access to the vascular channels and improve cellular integration as well as avoid adverse reaction to the residual organic material.

Effects of SCCO2 on Allograft Bone

The extraction characteristics of SCCO2 are valuable for processing of allograft bone. Fages et al (Fages et al., 1994b) were the first group to apply SCCO2 treatment to the processing of allograft tissue. In their seminal work, they investigated the effect of SCF treatment on both the composition and structure of bovine cancellous bone that was treated with either SCCO2 alone, SCCO2 followed by hydrogen peroxide (H2O2) extraction or SCCO2 followed by protease extraction.

Scanning Electron Microscopy (SEM) analysis showed that complete removal of lipids and cellular debris occurred after the combined SCCO2 treatment and hydrogen peroxide extraction. The results of this study were a promising first step in the adoption of SCF technology for allograft processing and sterilization. It confirmed that SCCO2 treatment could completely remove the lipid fraction of medullary tissue. Furthermore, following SCCO2 treatment the inherent micro porosity of bone was

Figure 1: Phase Diagram of Carbon Dioxide

Carbon Dioxide has the properties of both a gas and a liquid at the when it is in the Supercritical region.

This occurs at a pressure of 74 atmospheres and importantly at a temperature of 31.1 °C.

This temperature does not degrade the proteins!
shown to be better exposed, improving the effectiveness of subsequent extraction or processing steps in removing unwanted components.

Following on from this study Frayssinet et al (Frayssinet et al., 1995) performed an in vivo study to histologically evaluate the effects of SCCO2 on xenogeneic bone. In this study the authors utilized bovine femoral heads to manufacture cancellous bone cylinders. The bone structures were treated using a SCCO2 delipidation process followed by a H2O2 or protease extraction step. Treated cylinders were implanted into either femoral condyle or into tibial proximal epiphysis of the sheep for periods of 3 weeks, 2 months, and 4 months. Histological evaluation showed that partial deproteination and delipidation by SCCO2 treatment improved osteointegration and suppressed the inflammatory reaction compared to the untreated bone. The first step to implant incorporation i.e. direct mineralization at the material interface was also evident in the SCCO2 treated bone.

Frayssinet continued this research and investigated the effect of SCCO2 treatment on the biological response of allograft bone (Frayssinet et al., 1998). In this study, ovine cancellous cylinders were treated sequentially with SCCO2, H2O2, ethanol, followed by terminal sterilization
by gamma irradiation at 25kGy, and were then implanted into osseous defects in sheep. Control samples were only sterilized using gamma irradiation prior to implantation. The animal were sacrificed at 1, 4 or 8 months and both qualitative and quantitative histological analysis was performed. At 1 month, qualitative results showed significant lymphocyte and plasmocyte infiltration around the control samples compared to the SCCO2 treated group where there was osteoid apposition on the surface of the implanted graft. This trend continued at 4 months with the control grafts mostly osteolysed and replaced by connective tissue, while the SCCO2 treated grafts were synthesized at the graft-host interface, showing signs of remodeling/integration by creep substitution. These findings were reinforced by the quantitative results which showed statistically more bone formation in the SCCO2 group at all time points.

The results of this study advocate preservation of biological properties following SCCO2 treatment. The absence of osteoclastic resorption of the graft as seen in the control group, indicates the treatment used in this study effectively removed any antigenic factors from the graft resulting in a minimal immune reaction. Furthermore, the degree of graft-host integration would suggest that the osteoconductive properties of the graft were preserved following treatment.

**Terminal Sterilization**

SCCO2 is not only used for treatment of allograft bone but also for terminal sterilization. For a sterilization method to be approved by the FDA it must demonstrate a SAL level of 10-6, or at least six log reductions in bacterial spores and viruses. A number of studies have investigated the use of SCCO2 treatments for sterilizing both bacteria and viruses.

For SCCO2 sterilization procedures, active sterilants and co-solvents are utilized to improve the efficacy of the SCCO2 treatment and consequently minimize the experimental pressure, time and temperature required to inactivate the bacteria and viruses and preserve the biomechanical properties and biologic potential of allograft bone.
References: