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**FASTER OSSEOINTEGRATION AND VIRAL SAFETY OF BONE ALLOGRAFTS  
AFTER SUPERCRITICAL CO<sub>2</sub> EXTRACTION**

J. Fages, \*D. Mathon, †B.Poirier, P. Frayssinet, \*A. Autefage and †D. Larzul

Bioland, 132 route d'Espagne F-31100 Toulouse, France

\*ENVT, 23 chemin des Capelles F-31076 Toulouse, France

† Institut Pasteur Texcell, 25 rue du Dr Roux F-75015 Paris, France

A new bone tissue process using supercritical fluid (SCF) technology has recently been described (Fages *et al.* 1994).

Due to their unique properties of diffusivity, density and viscosity, supercritical fluids are used as selective extraction solvents. They are particularly adapted for extraction of components entrapped in a solid microporous matrix, which is the case of bone tissue.

Supercritical CO<sub>2</sub> extraction leads to a total range in-depth delipidation of the bone porosity. Three additional steps complete the bone processing: Hydrogen peroxide is used for protein and cellular debris elimination. Sodium hydroxide for the inactivation of potentially present prions, and ethanol for final cleansing and for its well-known virucidal properties.

Such treated allografts were implanted in sheep femur condyles and tibial epiphyses in comparison with non-treated allografts. Implantation times were 1, 4 and 8 months.

After one month, treated allografts trabeculae were already linked with those of the recipient bone tissue. At the external surface of the allograft trabeculae, new bone formation could be observed. This immature new bone tissue was edged by several layers of active osteoblasts. Some Howship lacunae were visible indicating an osteoclastic resorption.

Non-treated implants showed no link with the recipient tissue. A thick rich-cell conjunctive tissue was found between the implant and the recipient bone tissue. No sign of new bone apposition could be seen. Implant pores at the outer sides were filled with fibrous tissue containing lymphocytes and plasmocytes. Other pores were filled with necrotic tissue, remnant of the allogenic medullary tissue. A heavy resorption due to plurinucleated cells had begun. These cells had morphology of giant cells and not of osteoclasts.

After 4 months, osteointegration of treated allografts had progressed and was almost completed. Non-treated allografts were resorbed and replaced by a conjunctive tissue harbouring lymphocytes and activated macrophages. A few trabeculae fragments were integrated.

After 8 months treated allografts were completely integrated and the graft could no longer be distinguished. On the contrary, non-treated allografts were almost completely resorbed leaving cellular conjunctive tissue and necrotic zones within pores.

This experiment gives clear confirmation that this allograft treatment brings enhanced integration potential to the graft. A lowering of immunitary reactions linked with the removal of medullary cells and cellular components could be involved as well as an improved graft recolonisation due to the cleansing of the graft porosity.

The major concern of bone banking remains the risk of viral disease transmission. HIV-1 transmission by bone allografts has been reported twice while three cases of transmission of the hepatitis C virus by tissue transplantation have been published.

Despite an increasing safety level in bone banking procedures (donor screening, serological testing,...), a small but irreducible risk is always present. Moreover, there is still a large proportion of bone allografts which are just stored frozen. The most used sterilisation processes are:  $\beta$  or  $\gamma$  irradiation, ethylene oxide or autoclaving. None of these methods have proved to be totally satisfactory, either due to the resistance of some viruses to physico-chemical processing or due to the loss of inductive or mechanical properties.

One of the principal approaches to control potential viral contamination of biologicals is to test the capacity of the preparation process to remove or inactivate viruses. Each of these four processing stages have been checked for virus inactivation by spiking of a significant amount of a virus to the material and the determination of the virus removal or inactivation during the tested step. Four viruses chosen according to the EC guideline on virus inactivation validation (EC III/8115/89) were used:

- HIV-1 (RNA enveloped retrovirus)
- Sindbis virus (RNA enveloped virus, model for Hepatitis C virus)
- Polio Sabin virus (RNA non enveloped virus, model for Hepatitis A virus)
- Pseudorabies virus (DNA enveloped virus as the Hepatitis B virus).

The ability of each studied step to inactivate and/or eliminate the viruses was evaluated from two pieces of human femoral head treated independently. The samples obtained from the bone pieces, spiked and then treated or not were assayed for virus contents by infectivity measurements. The table below summarizes the reduction factors which are the log<sub>10</sub> of the ratio of the virus load before and after the treatment.

<i>virus</i> <i>stage</i>	<i>HIV-1</i>	<i>Sindbis</i>	<i>Polio</i> <i>Sabin</i>	<i>Pseudo</i> <i>rabies</i>
SC CO <sub>2</sub>	>4.05	>4.31	>6.58	>4.02
H <sub>2</sub> O <sub>2</sub>	>3.61	>4.89	>7.05	>4.71
NaOH	>2.19	4.08	4.25	>4.66
C <sub>2</sub> H <sub>5</sub> OH	>4.37	4.98	>6.60	>4.21
<i>Total</i>	>14.22	>18.25	>24.48	>17.50

These results clearly indicate that this process is efficient in eliminating or inactivating the tested viruses. It is proposed that this process could become a new way for processing bank bones alternatively or additionally to the procedures presently used.

## REFERENCES

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