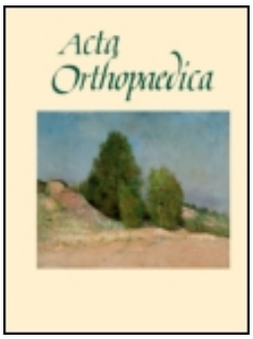

Acta Orthopaedica

ISSN: 1745-3674 (Print) 1745-3682 (Online) Journal homepage: informahealthcare.com/journals/iort20

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To cite this article: Suvi Haimi, Marko Wahlman, Mari Mannila, Vesa Virtanen & Martti Hirn (2008) Pulse-lavage washing is an effective method for defatting of morselized allograft bone in the operating theater, Acta Orthopaedica, 79:1, 94-97, DOI: 10.1080/17453670710014824

To link to this article: https://doi.org/10.1080/17453670710014824

Published online: 08 Jul 2009.

Pulse-lavage washing is an effective method for defatting of morselized allograft bone in the operating theater

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Submitted 07-03-03. Accepted 07-07-29

Background and purpose **Incorporation of fresh-frozen allograft bone and safety aspects associated with this procedure can be improved by removing blood and lipids from the bone. We investigated in a quantitative manner how efficient pulse lavage might be for removal of adipose tissue from morselized allograft bone.**

Methods **Depending on the study, the washing was performed with an average of 0.8 L or 1.6 L of sterile saline at room temperature. Fat content of the morselized bone samples was determined using hexane elution. The efficiency of pulse lavage alone was compared with that after an additional wash in 12 L of warm water (55°C). Unprocessed controls were also included for comparison.**

Results **Pulse lavage with 0.8 L saline alone removed 80% of the fat from the bone, whereas 95% of the fat was removed when washing was performed with 1.6 L of saline. The cleansing efficacy was improved further when an additional wash with warm water was used.**

Interpretation **Our results indicate that pulse-lavage washing alone at room temperature is an effective method for defatting of morselized allograft bone, but an additional wash with warm water improves the cleansing efficiency. Pulse lavage is easily available and simple to use in the operating theater.**

The use of allogenic bone in orthopedic surgery has increased steadily in recent years. The main source of allograft bone is femoral heads harvested from living donors during primary hip arthroplasties (Grieb et al. 2005, Yates et al. 2005). The transmission of viral diseases through transplantation of allografts has been a major concern (Tomford 1995, Aspenberg 1998). Most of the processing methods have been directed toward ensuring the safety of the patient by minimizing virus transmission (Grieb et al. 2005). The risk of infection is directly related to the amount of blood in the graft (Tomford 1995). It has been demonstrated that the risk of transmission of both HIV and HCV can be significantly reduced by removing blood and marrow from the bone (Tomford 1995, Yates et al 2005).

In addition, lipid extraction may increase the incorporation of frozen bone allografts and improve cup stability in impaction grafting (Thoren et al. 1995, Ullmark 2000, van der Donk et al. 2003, Arts et al. 2006). Pulse-lavage washing removes lipids and blood from morselized bone (Hirn 2001) and lowers the bacterial count in the graft (Hirn et al. 2004). Thus, it has been recommended that washing of morselized allograft bones in the operating theater should be done before transplantation. We evaluated in a quantitative manner how effectively pulse-lavage washing can remove lipids from morselized bone.

Material and methods

The grafts were obtained from the tissue bank of Tampere University Hospital. For the primary

DOI 10.1080/17453670710014824

Table 1. Primary study. Fat percentages (F%) of unprocessed and processed condyle samples and fat removal efficiencies of the different cleansing methods

Sample	Unprocessed F%	Pulse lavage (0.8 L) F%	Fat removal efficiency %	Pulse lavage (0.8 L) + warm water F%	Fat removal efficiency %
1/B	47	8	83	3	93
2/A	54	10	81	6	89
2/B	50	12	76	5	90
3	48	10	79	6	87
Mean	50	10	80	5	90

study, 5 condyles around the knee were harvested from 3 donors: 4 femoral condyles of 2 distal femurs and 1 proximal tibia. For the main study, 10 femoral heads from 10 living donors were selected. The femoral heads were retrieved in arthroplasty operations. Each of the donors was screened to be virus-negative according to the standards of the European Association of Tissue Banks (EATB and EAMST 1997). The condyles tested negative by bacteriological screening but femoral heads were rejected for different reasons, including positive bacterial culture. All bones were stored at –75°C before selection. Each of them was almost 5 years old. The bone grafts were cleaned free of remnants of chondral and soft tissue and then morselized separately using a bone mill (Howex, Gävle, Sweden). A standard 2-mm blade was used. This blade produced chips with a volume range of 0.3 × 0.3 × 0.3 mm (0.027 mm^3) to 2 × 4 × 15 mm (120 mm^3) as measured using a ruler. Condyles around the knee were morselized without cortical bone of the diaphysis. Femoral heads were ground with the cortical bone left in the neck of the femur.

Altogether, there were 15 morselized samples: 5 condyles for the primary study and 10 femoral heads for the main study. Each of these morselized samples was divided into 3 groups. The first group was left as an unprocessed control. The second group was washed by pulse lavage with physiological saline at room temperature. In the primary study, approximately 0.8 L of physiological saline was used per sample. In the main femoral head study, the amount of saline was doubled to 1.6 L per sample in order to determine whether increasing the amount of saline would improve the results. The pulse- lavage washing was done as previously described (Hirn 2001). Briefly, the morselized chips were washed by placing them in a sieve, where the material was spread out in a thick layer on the bottom. A MicroAire Pulse Lavage unit (4740; MicroAire Surgical Instruments, Charlottesville, VA) with an output pressure of up to 0.14 MPa was used. The third group of samples was treated like the second one, but after pulse lavage it was washed again in the sieve, this time under running tap water at 55°C for 1 min (12 L).

After washing, the samples and the controls were frozen at –75°C and stored for 4 weeks before determination of residual fat content by ultrasound-aided hexane extraction. The samples were freeze-dried before the lipids were extracted in hexane. The hexane was evaporated and the resultant residual weight was expressed as a percentage of the (extracted original) dried cancellous bone powder weight.

Statistics

The results were compared using paired t-test. A p-value of < 0.05 was considered significant. The analyses were done with SPSS version 13.

Results

In the primary study, the average efficiency of pulse lavage washing (0.8 L) alone in removing fat from morselized bone samples was 80%. The average efficiency rose to 95% when 1.6 L of saline was used. In both studies, the additional warm water wash improved the results considerably (Table 1). In the main study, the difference in efficiency between pulse lavage alone and pulse lavage with warm water wash was found to be statistically significant ($p = 0.008$) (Table 2).

Table 2. Main study. Fat percentages (F%) of unprocessed and processed femoral head samples and fat removal efficiencies of the different cleansing methods. The difference in fat removal efficiency between samples washed with saline pulse lavage (1.6 L) and samples washed with saline pulse lavage and additional warm water was statistically significant (p = 0.008)

Femoral head	Unprocessed F%	Pulse lavage (1.6 L) F%	Fat removal efficiency %	Pulse lavage (1.6 L) + warm water F%	Fat removal efficiency %
1	36	0.1	100	0.5	99
2	57	0.8	99	0.7	99
3	40	1.9	95	0.3	99
4	43	1.4	97	0.7	98
5	52	5.9	89	1.6	97
6	42	3.8	91	0.7	98
7	49	3.4	93	2.0	96
8	55	2.7	95	1.5	97
9	42	1.6	96	0.5	99
10	50	2.1	96	0.5	99
Mean	47	2.4	95	0.9	98

Discussion

In many countries most allograft bone is used as unprocessed fresh-frozen bone (Hirn 2001, Yates et al. 2005). The most usual form of allograft bone is morselized femoral head, which appears safe to use—both virologically and bacteriologically (Aspenberg 1998, Hirn 2001). Femoral heads have many advantages over allograft bone from cadavers (Aspenberg 1998). Living donors can be double-tested for viruses, their medical history is more reliable, and generally speaking they represent an age group with low-risk activity (regarding lifestyle).

Pulse-lavage washing lowers the bacterial bioburden considerably (Hirn et al. 2001). At the same time, it also removes lipids and other loose material from the bone. Experimental studies have shown that incorporation of lipid-extracted bone is better than using a graft that has not been defatted (Thoren et al. 1995, van der Donk et al. 2003). Defatting does not appear to alter the mechanical properties of the graft (Thoren et al. 1995). On the contrary, washing of the graft has been shown to increase cup stability in impaction grafting (Ullmark 2000, Arts et al. 2006). According to these reports, the size of the morselized graft also matters. Larger bone graft size enhances cup stability. Different washing systems were used in the two studies, but the lipid extraction capabilities of these systems were not evaluated (Ullmark 2000, Arts et al. 2006). In our study, the efficacy of washing to remove lipids was comparable to that of some other more complex methods (Lomas et al. 2000). The effect of pulse-lavage washing was considerably improved by using more saline. Also, washing with warm water (55°C) improved the result, but it complicates the procedure somewhat and can be omitted. Also, high temperatures may have a negative effect on bone incorporation. Pulse-lavage washing alone seems to be adequate for removal of fat from morselized bone.

Many bone banks use quite costly and time-consuming chemical and physical cleansing procedures. These methods are meant for whole femoral heads or big bone blocks, and not for morselized bone (Lomas et al. 2000, DePaula et al. 2005, Yates et al. 2005). For example, Lomas et al. (2000) reported that the average efficiency of fat removal from whole femoral heads was 75% with the processing method they used. In our study, the average fat removal efficiency from morselized bone was 95% when 1.6 L of pulse saline was used. Thus, our cheaper and time-saving method can be recommended for cases where morselized bone is used. There was no visible difference in the color of the graft after washing with 0.8 L or 1.6 L saline. It seems that pulse-lavage washing with the standard 1-L saline bag allows adequate fat removal from an average morselized femoral head. The effect can be improved to some degree by washing the graft slightly longer after the point at which the color of

the morselized material has turned white. Nevertheless, pulse-lavage washing cannot be considered to be an effective method for larger bone blocks and whole femoral heads. Its effect in removing fat and lowering bacterial bioburden is mostly limited to the surface of the bone (Hirn et al. 2004).

We did not measure the cleansing efficacy for blood products, but washing changed the color of the morselized bone material from reddish to white, as previously reported (Hirn 2001). Thus, it appears to remove most of the blood cells from the ground bone and must therefore also improve the safety from a virological standpoint. However, irradiation and heavy chemical processing methods are needed for reliable virological and bacteriological safety. Unfortunately, these methods have a negative effect on the biological and mechanical properties of allograft bone (Currey et al. 1997, Boyce et al. 1999). Pulse-lavage washing does not have such an effect, yet its efficacy in removing fat and other remnants unnecessary or even harmful for bone incorporation is certainly significant. The method we used is cheap, quick, and available in every hospital. Still, it must be emphasized that neither pulse lavage nor any other washing method can obviate the need for rigorous donor selection and laboratory screening for viruses and bacteria.

Contributions of authors

SH and MH planned the study, carried out the experimental work, and wrote the manuscript. MH also supervised the study. MW participated in the experimental work. VV and MM analyzed the results on residual fat content.

Special thanks to Heini Huhtala for statistical advice and to Miia Juntunen for valuable laboratory assistance. This research was supported by the competitive research funding of the Pirkanmaa Hospital District and the City of Tampere.

No competing interests declared.

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