Cancellous bone homograft storage with aluminium-polyethylene bags

A. Meana1,*, R. Martinez1, P. Cañal1, M.J. Arriaga1, F. San Román1, S. Llames1, C. Orós2, A. Moreno2 and C. Fernandez1

1Centro Comunitario de Sangre y Tejidos, Principado de Asturias, Spain; 2Department of Infectious Diseases, Hospital Central de Asturias, Spain; *Author for correspondence (e-mail:investigacion@c-transfusion-asturias.com; phone: +34985232426; fax: +34985240038)

Received 5 March 2004; accepted in revised form 26 August 2004

Key words: Aluminium polyethylene bags, Bone homografts, Cryopreservation, Storage, Tissue bank

Abstract

In order to transport and cryopreserve human tissues, it is essential to have an easy-to-use recipient where tissues can be kept in sterile conditions. Here we show the results obtained by using Macopharma’s tissue freezing bags, an aluminium-polyethylene multilayer bag, in our tissue bank of the Centro Comunitario de Sangre y Tejidos de Asturias. Five hundred and twenty-seven cancellous bone homografts were obtained from hospitals located 120 km around our Bank. The homografts were submitted to bacteriological controls and sent to our bank in these bags. They were stored at −70 °C and sent in dry ice to about 50 hospitals, where the tissue was bacteriologically controlled and grafted. Furthermore, the behaviour of these bags at −140 °C (vapour nitrogen) or −196 °C (liquid nitrogen) was tested. Our results indicate that Macopharma aluminium-polyethylene bags are suitable for the transporting and cryopreserving of cancellous bone homografts. These bags could also be used for keeping tissues in nitrogen containers.

Introduction

Musculoskeletal tissue is the most frequently transplanted human tissue and cancellous bone represents more than 50% of the musculoskeletal tissue grafted. During 2002, 8655 patients were grafted in Spain with this tissue (Organización Nacional de Trasplantes 2003). This fact makes it necessary to obtain the largest quantity of tissue in the best conditions in order to meet surgical needs. Femoral heads, coming from living donors undergoing hip prosthesis surgery, are one of the main sources of cancellous bone (Tomford and Mankin 1999). Ideally, sterile bone should be obtained in an easy way in the surgical theatre, bacteriologically tested and sent to the bank in a container that could preserve the sample from contamination. Moreover, this container must be easy to handle and able to support storage and freezing conditions in the tissue bank. These conditions must also be kept when sending back the piece from the tissue bank to hospitals for grafting. However, the hospitals where they come from are not always located near the tissue bank. In the Centro Comunitario de Sangre y Tejidos de Asturias, we receive and send femoral heads to diverse hospitals of the country whose distances from the bank range from 1 to 120 km. From January 2000, we have used Macopharma’s tissue freezing bags, an aluminium-polyethylene multilayer bag, for the transport and storage of femoral heads. In this paper, we describe the results we
have obtained when using these bags in the collection, transport, storage and grafting of cancellous bone. Also, bacteriological security tests in extreme storage conditions (vapour or liquid nitrogen) have been performed in these bags.

Material and methods

Obtaining cancellous bone

Five hundred and twenty-seven cancellous bone homografts were obtained from the surgical theatre of nine hospitals by means of a collection kit consisting of the following: a Macopharma aluminium-polyethylene bag (sterilized by ethylene oxide and protected by a double envelope system), two sterile containers, a sterile swab for bacterial control, a tube for blood collection (serological studies) and the papers for the donor’s data (Figure 1). In order to control its sterility, the femoral head was laid on a sterile tray and a small bone fragment was taken. Then, the bone piece was washed (×5) with saline. The last wash was collected and finally the bone surface was swabbed. Afterwards, the femoral head was introduced by the surgeon in the Macopharma aluminium-polyethylene bag. This aluminium bag was closed by rolling it up and then introduced inside the second sterilized bag. The femoral head, the bacteriological and serological samples as well as the documentation were sent to our bank in an insulated container. Once arrived at the bank, the Macopharma aluminium-polyethylene bag was definitively closed by thermal sealing and kept in an electrical freezer at −70 °C until use. The average period of storing the bags at this temperature was 3 months, although they could be stored for up to 5 years.

Bacteriological and serological tests

Bacteriological samples obtained from femoral heads underwent the following tests: the bone fragment was inoculated in thioglycollate medium. The swab was inoculated in thioglycollate medium and later seeded on EMB Levine, Columbia CNA and Blood Agar medium. The bone wash was centrifuged at 3000 rpm and the pellet was seeded on Chocolate Blood Agar. Samples were incubated at 35 °C in a 5% CO₂ incubator for 48 h. A cancellous bone homograft was considered sterile when all bacteriological cultures performed were negative. The blood sample was tested for HIV, Hepatitis B and C virus and syphilis.

Grafting the cancellous bone

The cancellous bone homografts were sent in an isothermal container to those hospitals located at less than 20 km from our bank. When larger distances were to be covered, they were sent in dry ice. After thawing, the integrity of the bags was checked, the femoral head was removed and placed in a sterile tray where it was washed in saline. This wash was used as a bacteriological pre-grafting control.

Behaviour of Macopharma aluminium-polyethylene bags in extreme storage conditions

Sterile cryovials were introduced inside aluminium polyethylene bags and closed by thermo sealing. The bags were stored in a nitrogen container, 3 bags in the vapour phase (at −130 °C) and three in