■ THA基礎，他

バーコードシステムによる同種骨管理

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はじめに

著者らは，2000年に，同種骨の供給および支給システムである骨バンクネットワーク（Bone Bank Network，以下BBN）を設立した[1]．さらに，より安定したシステムとするために，2005年にNPO法人骨バンクネットワーク東海を設立した．BBNの設立目的は，1）手術時に不要となる骨を提供することにより同種骨移植術に有効利用できることにする，2）摘出骨を廃棄している病院から，必要としている病院へ機能的に供給する，3）摘出同種骨の品質を一定化することである[2]．

2002年から2009年の8年間に骨バンクへ供給された大腿骨頭は，合計985個，骨バンクから支給された大腿骨頭は合計889個で，廃棄された骨頭は合計35個であった．専用冷凍庫で，常時50個程度の大腿骨頭が保存できた（図1）．

骨頭の廃棄理由は，培養陽性4個，ドナーのMRSA感染判明1個，ドナーの白血球10000以上1個，HTLV-1陽性4個，HTLV-1/HIV未検査5個，術中不要/支給後返却5個，−20℃放置1個，インスリン治療歴あり1個，同意書なし1個，搬入登録無し4個，個人用2個，詳細不明6個であった．

これらの活動から，骨バンク運営における問題点は次の5項目であった．

①個人情報守秘化，②供給体制，③支給体制，④管理体制，⑤トレーサビリティ

以上の問題点を改善するため，バーコードを利用した新たな管理方法を導入することとした．

目　的

本研究の目的は，骨バンクの活動状況の報告をすることと，同種骨の安全性と有効性を高めるために，バーコードシステムによる管理方法を開発することである．

方　法

1）バーコード作成（図2）

バーコードは医療材料の管理に一般的に使用されているコード128を用い，15ケタの数値の骨バンクプライベートコードとした．あらかじめコードを決め，骨頭情報を入力することでバーコードデータ化した．内容は　①重複防止のための識別用番号，②摘出日，③病院番号，④管理者番号，⑤原疾患，⑥軟骨除去の有無，⑦重量と定義した．

2）バーコード登録管理システム（図3）

骨頭は，摘出病院で既往症・感染症検査を記入し，速やかに骨バンクへ輸送される．記入情報に不備があれば廃棄とする．データベース上への登録によって，固有ID番号が作成され，バーコードラベルが発行される．骨頭は，バーコードラベルとともに−80℃の冷凍庫に保管する．登録の段階で，未検査の感染症があれば，添付された血清で，骨バンクから検査依頼し，別に保管する．感染症陽性の場合は廃棄，陰性であれば保管継続とする．摘出から3ヶ月間保管された骨頭を支給可能とする．

3）バーコード支給システム（図4）

骨頭の依頼を受けて，骨バンクスタッフが，データベース上で適切な骨頭を選択する．そして保管庫より，目指す骨頭を取り出し，バーコードスキャンを行って骨頭を確認

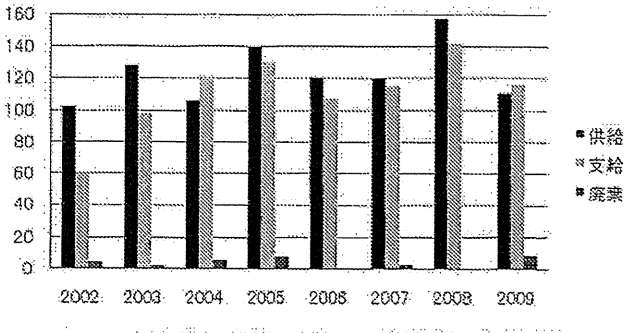

図1　大腿骨頭の供給・支給および廃棄数の年次推移

図2　バーコード作成

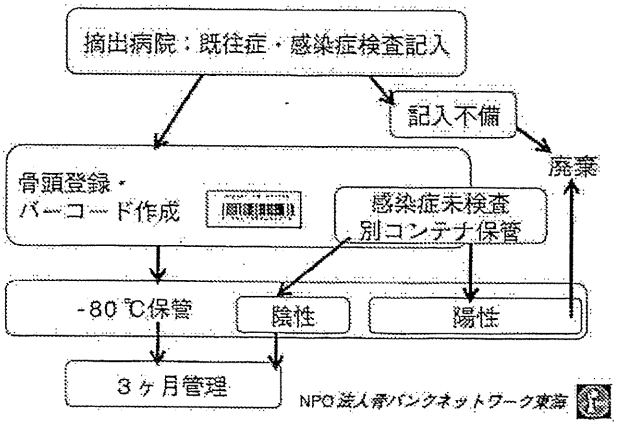

図3　バーコード登録管理システム

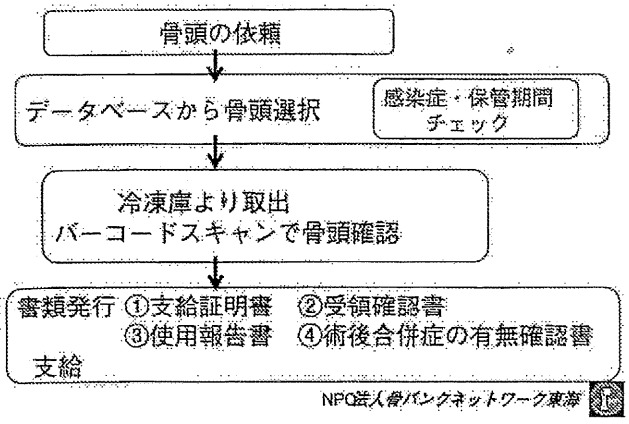

図4 バーコード支給システム

する．支給時の発行書類として，支給証明書，受領確認書，使用報告書の3書式を作製する．

骨頭使用後は，使用報告書を用いて，手術内容，日時，病院，患者IDを，使用骨頭のバーコードラベルとともに，骨バンクへ報告することを義務付けた．また，追跡調査のために，術後合併症の有無確認書を作成，後日提出を依頼することとした．

結　果

本バーコードシステムの活用と，支給証明・受領確認書発行，使用報告書提出によって，従来から問題となっていた点を改善できると考えている．2010年1月より，本システムの稼働を開始し，現在計68個の骨頭をバーコード登録，4施設へ計27個の骨頭を支給，2施設より計12個についての使用報告を受理した．

考　察

本バーコードシステムによって5つの問題点が改善される．

① 個人情報守秘化：バーコードラベルにより，個人情報表示を除去して提供できる．

② 供給体制：バーコード発行で登録が明確となる．

③ 支給体制：バーコードスキャンにより簡便に支給管理ができ，さらに支給証明・受領確認の書類発行で流通が明確となる．

④ 管理体制：バーコードスキャンとデータベース機能で不良在庫の発生を防止できる．

⑤ トレーサビリティ：骨頭使用の際，バーコードスキャンで固有IDを電子カルテに簡易保存でき，また，報告書類の発行で追跡調査ができる．

本システムには，改善すべき点もある．供給病院からの搬入は，摘出後48時間以内に搬入されていないものが多く，−20℃の冷凍庫に保存した場合マニュアル違反となる．今後の課題として，搬入方法・経路の確立が求められる．また，骨バンクの専用冷凍庫に空きが無い場合は，一時的に共用冷凍庫での保管となる．骨頭の需要は今後も高まることが予想され，安定供給のために専用冷凍庫の容量を大きくする必要がある．供給骨頭の適性確認，支給における患者の優先順位や適合性判定をするコーディネーターがいないことも問題である[3]．供給・支給を円滑に進めるうえで，関連施設との連携を強化し，本システムの有効性を高めるためにも，コーディネーターの育成が急務である．

まとめ

骨バンクの運営における個人情報守秘化，供給体制，支給体制，管理体制，トレーサビリティの問題点を解決するために，バーコードシステムによる管理方法を開発した．

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Virus inactivation in bone tissue transplants (femoral heads) by moist heat with the 'Marburg bone bank system'

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Abstract

Several virus inactivation procedures like heat treatment, gamma irradiation and chemical sterilization are used to increase the safety of bone tissue transplants. In this study we present data on the virus-inactivating effect of heat disinfection on human femoral heads, using the Marburg bone bank system 'Lobator sd-2'. Three enveloped viruses (human immunodeficiency virus type 2 [HIV-2], bovine viral diarrhoea virus as a model for Hepatitis C virus [HCV], and the herpesvirus pseudorabies virus), and three non-enveloped viruses (hepatitis A virus, poliomyelitis virus, and bovine parvovirus) were investigated.

In a model system the central part of human femoral heads was contaminated with the respective cell-free virus suspension, establishing a direct contact between virus and native bone tissue. The core temperature in the femoral heads during the sterilization process was determined in additional model experiments. A temperature of 82.5 °C, given by the manufacturer as the effective temperature for virus inactivation, was maintained for at least 15 min in decartilaged femoral heads with a diameter of $\leq$56 mm. Heat treatment using the Lobator sd-2 inactivated all viruses in human femoral heads below the detection limit (at least by a factor of ≥ 4 $\log_{10}$).

By combining a well-focussed anamnesis of the donors and serological testing for relevant infection markers (anti-HIV-1/2, HBsAg, anti-HBcore, anti-HCV, TPHA) with heat treatment of femoral heads in the Lobator sd-2 system, a high safety level is achieved. To further increase virus safety of cadaveric bone transplants, it is recommended that multi-organ donors are tested by nucleic acid testing (i.e. polymerase chain reaction) for HIV, HBV and HCV genome.

Keywords: Bone tissue transplants; Transplantation safety; Virus inactivation; Thermal disinfection; Lobator system

1. Introduction

In spite of increasing efforts to develop alternative materials and procedures, allogeneic bone transplantation remains an indispensable tool for reconstructing extensive bone defects. Bone transplants are particularly used in surgery to exchange endoprostheses in the hip joint, in operations of the spinal column and for reconstruction after extensive tumour resection. In Germany 71,000 autologous and 25,000 allogeneic bone transplantations are performed each year with an additional need for approximately 18,000 transplants [1]. In the USA the number of allogeneic bone grafts implanted amounts to between 300,000 and 400,000 per year [2].

In addition to clinical or functional aspects, the risk of transmitting pathogens via the transplant is of major concern [3,4]. Transmission of viruses and of microbial pathogens by bone tissue has been reported [5,6]. However, there are no reports on the transmission of agents inducing TSEs like Creutzfeldt-Jakob disease by

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allogeneic bone transplants, implying an extremely low risk for the transmission of TSE agents via bone tissue [7].

As starting material for the production of bone transplants human bone tissues are collected from extremity bones like femoral head and tibial plateau, vertebral body and Os ilium. Femoral heads are especially suitable for correction of malpositions as well as for individual adaptation of transplants in endoprosthetic surgery, because they can be fitted perfectly in size and shape, providing solid support.

Special requirements must be met to provide pathogen-free tissue. In addition to their reconstitutive efficacy, a central issue in quality assessment must be the validation of the disinfection or sterilization of the tissues. According to the guidelines for managing bone banks by the Bundesärztekammer (German Medical Association), only allogeneic bones or bone-derived materials subjected to validated inactivation procedures can be used for transplantation in cases where no testing of the donor is possible after a sufficiently long quarantine [8].

At present, several procedures for the inactivation of viruses such as heat treatment [9,10], gamma irradiation [11,12], and peracetic acid–ethanol treatment [13–15] are used in the production of bone tissue transplants. In Germany a common method used is heat treatment of femoral heads in the Lobator sd-2 system (telos, Marburg, Germany). In contrast to other pathogen-reducing treatment, thermophysical disinfection preserves favourable biological properties of the bone [10,16]. Although previous validation studies of the procedure have met the requirements of national and international standards [10,17–21], systematic investigations of the disinfection capacity of the Lobator sd-2 system are lacking.

It was the aim of this investigation to validate the virus-inactivating effect of the Lobator sd-2 system, using virus-contaminated human femoral heads. We developed a model system for the validation of the inactivation procedure, imitating in vivo conditions. The centre of human femoral heads was contaminated with a high-titre cell-free virus suspension, establishing a direct contact between virus and bone. This was of special interest since in previous investigations PCR tubes containing the virus suspension have been placed into the centre of the femoral head. In such a model system it is not possible to determine the influence of blood and bone components on virus stability [22].

2. Material and methods

2.1. Viruses

The selection of viruses was made according to the recommendations of the Paul-Ehrlich-Institut and the Bundesinstitut für Arzneimittel und Medizinprodukte [17]. The following viruses were investigated.

2.1.1. Enveloped viruses

Human immunodeficiency virus type 2 (HIV-2), ssRNA, Retroviridae, isolate SBL6669—propagated and titrated in Molt 4 clone 8 cells. This virus is a model for HIV-1. It is classified as having a low resistance against physico-chemical treatment and is inactivated at 60 °C [19,20,22].

Bovine viral diarrhoea virus (BVDV), ssRNA, Flaviviridae, strain Ug 59/Denmark as model virus for hepatitis C virus (HCV)—BVDV was grown in foetal calf cells. BVDV and HCV both belong to the Flaviviridae and have comparable physico-chemical properties (personal communication Central Laboratory of the Netherlands, Transfusion Service, Department Clinical Viro-Immunology [CLB] and Ref. [19]).

Pseudorabies virus (PRV; Aujeszky's disease virus of swine), dsDNA, Herpesviridae, strain Bartha—grown in mink lung cells. PRV is used as a model for human herpesviruses. It is considered to be moderately resistant to heat treatment [19].

2.1.2. Non-enveloped viruses

Hepatitis A virus (HAV), ssRNA, Picornaviridae, strain HM 175cyt—grown in embryonal rhesus monkey kidney cells, CRL 1688. HAV is considered to be highly resistant to heat treatment [19].

Poliomyelitis virus type 1 (PV-1), ssRNA, Picornaviridae, vaccine strain PI 18—grown in human lung cells. PV-1 is considered to be moderately resistant to heat treatment [19]. After 30 min at 60 °C complete inactivation has been demonstrated [22].

Bovine parvovirus (BPV), ssDNA, Parvoviridae—BPV is a small virus (about 20 nm) with icosaedral symmetry. It can be grown in calf lung cells and is widely accepted as a model for human parvovirus B19 because of its very high resistance to heat (personal communication CLB, The Netherlands and Ref. [19]).

2.2. Cell culturing and virus titration

Cell culturing as well as virus propagation and virus titration were performed in a laminar airflow safety cabinet essentially as described elsewhere [13,23].

2.3. Bone material (femoral heads)

The preparation of the femoral heads used in the present validation studies followed the procedures routinely used for the production of medicinal products at the bone bank of the university clinic Charité (Berlin, Germany). The bone donors were negative for HIV-1/2, HCV, HBsAg and TPHA in serological testing. The femoral heads generally had a diameter of 55 ± 1 mm and were stored at -70 °C until use.

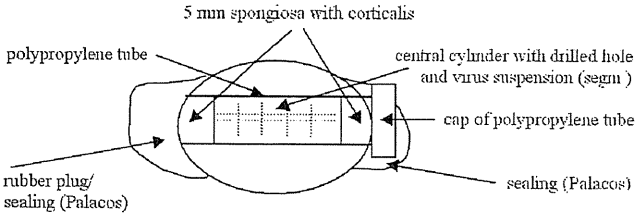

Fig. 1. Schematic drawing of the femoral head prepared for the validation study.

Human femoral heads were removed from the freezer (−70 °C) and thawed in double-distilled water at 20 °C. The tissue was inspected for visible damage, especially for necroses. Intact tissue was decartilaged, i.e. cartilage was manually removed by means of scalpel and bone rasp or cartilage milling cutter (Aesculap, Tuttlingen, Germany). To prepare the bone tissue for the validation of the heat treatment, it was processed as shown schematically in Fig. 1.

Through the centre of the decartilaged human femoral head, starting at the sagittal plane of the femoral head, a bone cylinder (∅ 15 mm) was sawed by means of a keyhole saw under constant cooling. The resulting channel through the femoral head was widened to a diameter of 17 mm to fit in a 15 ml polypropylene centrifuge tube with an outer diameter of 17 mm and an inner diameter of 15 mm (Nunc, Wiesbaden, Germany). The bottom part of the 120 mm long tube was cut off to fit the length of the channel. At one end of the bone cylinder a 5 mm disc was sawed off and used later as lid. Into the remaining cylinder a central hole with a length of 45 mm was drilled (diameter 6 mm), while a bottom plane of 5 mm remained intact. The drillhole served as container for virus suspensions. For technical reasons, permitting the further processing of the bone cylinder in the Omni-Mixer (type OM, Ivan Sorvall Inc., Norwalk, CT), the cylinder was sliced into discs of around 5 mm thickness.

The open bottom end of the polypropylene tube was closed tightly with a rubber plug which was sealed and fixed with Palacos-R bone cement (Heraeus Kulzer, Wehrheim, Germany). Then the unpierced part of the bone cylinder and the pierced cylinder disks were placed into the tube. After the drillhole was filled with 1 ml of the respective virus suspension, the bone lid was placed on top. Finally the tube cap was screwed onto the tube. The femoral head thus prepared for the experiments was placed into the sterile container of the Lobator sd-2 and heat treated according to the manufacturer's instructions (see below) in Ringer's solution (B. Braun, Melsungen, Germany). After the cooling phase the virus suspension was harvested from the drillhole under sterile conditions and the virus titre determined. The central bone cylinder as well as the suspension remaining in the tube were collected, 9 ml of cell culture medium were added and the bones in the medium were homogenized in a sterile stainless steel container of an Omni mixer in an ice-water bath at 1500 U/min. After centrifugation of the mixture (4 °C, 3000 ×*g*) the virus titres in the supernatant as well as in the suspension from the bone pellet were determined.

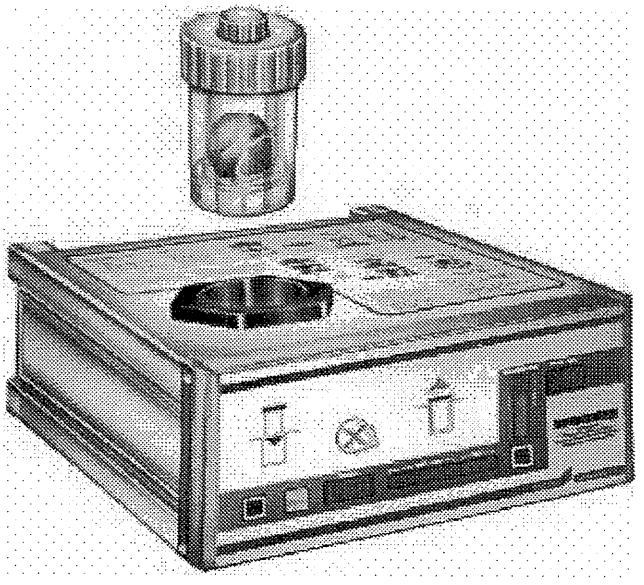

Fig. 2. Marburg bone bank system Lobator sd-2.

2.4. Lobator sd-2 system

This device was developed by telos H+V GmbH, Marburg, Germany, for thermal disinfection of allogeneic femoral heads for internal use in clinics (see Fig. 2).

The temperature gradients (heating phase, plateau, cooling phase) in the incubation device are programmed by the manufacturer and cannot be altered by the user. According to the manufacturer, a temperature of 82.5 °C for at least 15 min is reached in the centre of femoral heads with a diameter of ≤56 mm. For technical details of the calibration and validation of the device, see documentation of the manufacturer (http://www.telos1.de).

2.5. Evaluation of temperature kinetics by determining the core temperature in the femoral heads

To verify that the required core temperature and holding time were achieved, three additional experimental approaches were taken: native femoral heads (I), femoral heads with the cartilage removed (II) and the model system for the validation of virus inactivation (III; see Table 3). Starting at the onset of the *Ligamentum capitis femoris* (native or decartilaged), a canal with a diameter of 0.5 mm was drilled, ending in the centre of

Table 1
Determination of temperature kinetics in the femoral heads

Diameter of the femoral heads (mm)	Duration of incubation at ≥82.5 °C (min)	Peak temperature (°C)	Time necessary to reach 82.5 °C (min)
I: native			
52	26	85.7	53
53	20	84.7	54
61	–[a]	81.9[a]	–[a]
II: decartilaged			
51	31	88.3	48
52	32	86.2	49
53	29	87.2	46
53	24	85.7	53
54	21	84.7	53
54	23	84.6	54
56	24	85.3	50
61	15	83.8	64
III: model system			
51	23	85.3	57
53	21	84.7	55
53	19	84.2	56
55	28	85.9	50
56	20	83.8	59
58	18	85.4	56
61	10	82.8	61

[a] Required temperature was not reached.

the femoral head (i.e. for a femoral head with 55 mm ∅: 0.5 × 27.5 mm). A temperature sensor measuring at its tip was inserted, allowing only the central temperature to be recorded (T430-2L050, measuring from −40 to +500 °C, resolution/accuracy 0.5 K; Therm 2281-8, Ahlborn Mess- und Regelungstechnik, Holzkirchen, Germany). The sensor was fixed in the bone at the central position (i.e. in the example mentioned at 27.5 mm) with Palacos-R bone cement (Heraeus Kulzer). The wire of the temperature sensor was run through the screw-type cap of the disinfection container. The heat treatment in the polypropylene disinfection vessel followed the procedure described for the treatment of femoral heads. Temperature was recorded manually per minute and entered in a computer programme (off-line version 4.32/DOS-7.10, DEMA-soft GmbH, Holzkirchen, Germany).

3. Results

3.1. Core temperature measurements

The starting temperature within the femoral head ranged between 24 and 26 °C. It could be shown that the respective diameter as well as the type of manipulation played a role in the kinetics of the core temperature during the heating of the device (Table 1). Furthermore, difference in heat conductivity of individual bone tissues might influence the required heating time and the height of the peak temperature. As expected, the polypropylene tube inserted into the bone tissue in the model system had a retarding effect on the temperature kinetics as well as on the peak temperature achieved in the centre of the femoral head. However, in all experiments evaluating the virus model system (Table 1, III) with femoral heads ranging in diameter between 51 and 56 mm the temperature of ≥82.5 °C as well as the incubation time of at least 15 min were achieved.

In Fig. 3 the temperature kinetics in femoral heads with diameters of 51, 53 and 56 mm, respectively, are given. Despite a lower starting temperature the rise in temperature in the femoral heads was steeper in the heating period compared to the model system. Furthermore, in the native and the decartilaged femoral heads a higher peak temperature and a longer holding time at 82.5 °C were achieved. The analysis of the cooling period showed that the temperature decline in the femoral heads was faster than that in the model system (Fig. 3). However, in general the temperature kinetics in the femoral heads and in the model system were comparable.

To be able to compare the results of the temperature kinetics determined even more precisely, the area below the curves in Fig. 3 representing the temperature kinetics was calculated which gives a relative measure of the amount of heat. The areas between the x-axis (time in min) and the y-axis (temperature in °C) regarding model system and decartilaged femoral head were calculated as follows:

$$\text{Unit of area} = (x_{i+1} - x_i) - \frac{(y_{i+1} - y_i)}{2}$$

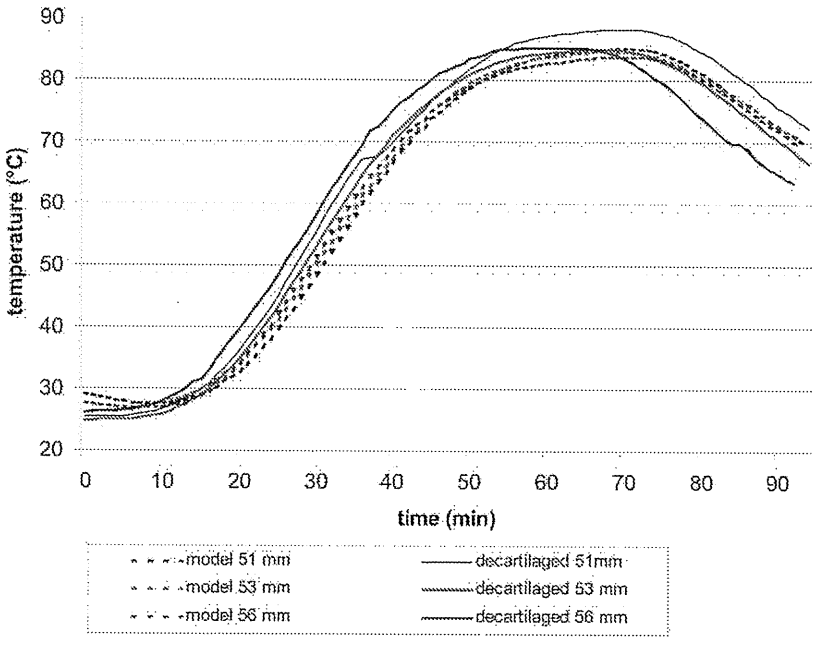

Fig. 3. Temperature kinetics in femoral heads.

Table 2
Relative heat quantities (temperature/time in units of area) in relation to the femoral head diameter

Relative quantity of heat (in units of area)	Femoral head ∅ (in mm)		
	51	53	56
Decartilaged femoral head	6123.30	5901.95	5892.55
Model system	5842.55	5832.25	5750.65

where x_i is time at point i and y_i is temperature at point i.

The results in Table 2 indicate that in the course of the disinfection process a larger amount of heat acts in the centre of the native decartilaged femoral head than in the centre of the model system.

In Fig. 3 the time interval is given when the temperature is $\geq$82.5 °C in relation to the diameter of the femoral head. The manufacturer declares that this interval marks the effective phase of inactivation. In the model system a shorter interval with a temperature above 82.5 °C is observed compared to the native or decartilaged femoral head. However, in all the native or decartilaged femoral heads as well as the models with a diameter of up to 58 mm the required temperature of 82.5 °C was reached and maintained for the necessary 15 min. Exceptions were the model femoral head with a diameter of 61 mm in which only a temperature of 82.5 °C for 10 min was maintained and the native femoral head in which only a peak temperature of 81.9 °C was reached (see Table 1).

3.2. Virus inactivation

For each experiment two femoral heads with an identical diameter (54–56 mm) were prepared. Both were spiked with a given virus suspension, then one femoral head was submitted to the disinfection procedure in the Lobator and the other was incubated at room temperature (RT) and at 4 °C, respectively, as controls to determine the influence of the bone material and storage conditions on the virus inactivation. Three independent experiments were performed for each virus. In two experiments the incubation of the controls was performed at RT (20–25 °C, see Table 3). In a third experiment the incubation of the controls was done at 4 °C (see Table 4).

The inactivation studies showed that in the disinfection device all the viruses were inactivated below the level of detection, i.e. the application of the Lobator sd-2 programme led to a virus reduction of more than 4 $\log_{10}$ in the core of human femoral heads. The controls incubated in parallel at RT showed very high spontaneous inactivation, particularly for HIV-2. When the controls were incubated at 4 °C, no or only a slight effect on the virus titres was observed.

In some of the experiments—probably depending on the biological and structural properties of the femoral head used—toxic effects of the suspension on the indicator cells were observed after incubation for 94 min in the Lobator sd-2 system. As summarized in Table 3, this resulted in a higher detection limit ($TCID_{50}$ given as $\log_{10}$), i.e. $\leq$2.49 (BPV, PRV, BVDV, HAV), respectively. Furthermore, in the PV-1 inactivation experiments the virus suspensions as well as the homogenates of the bone cylinders had a toxic effect on cells, and in these suspensions virus titres could not be determined (Table 3). In no other experiment in the Lobator sd-2 was toxic effects observed.

Table 3
Virus inactivation experiments in the Lobator sd-2 (Lsd-2) with a control at RT (values are given as $\log_{10}$)

Virus	Starting titre ($TCID_{50}$/ml)	Virus titre 94 min/RT ($TCID_{50}$/ml)	Virus titre 94 min/Lsd-2 ($TCID_{50}$/ml)	Reduction factor 94 min/RT ($TCID_{50}$/ml)	Reduction factor 94 min/Lsd-2 ($TCID_{50}$/ml)
BPV	8.49	4.74	≤2.49	3.75	≥6.00
BPV	8.25	5.74	≤2.49	2.51	≥5.76
BPV	7.49	3.74	≤1.49	3.75	≥6.00
PRV	8.00	4.49	≤2.49	3.51	≥5.51
PRV	8.37	6.25	≤1.49	2.12	≥6.88
PV-1	10.38	7.87	n.e.p.	2.51	n.e.p.
PV-1	9.87	7.37	n.e.p.	2.50	n.e.p.
HIV-2	5.55	≤1.49	≤1.49	≥4.06	≥4.06
HIV-2	5.00	≤1.49	≤1.49	≥3.51	≥3.51
BVDV	6.74	5.49	≤2.49	1.25	≥4.25
BVDV	6.62	5.25	≤2.49	1.37	≥4.13
HAV	7.40	4.88	≤1.49	2.52	≥5.91
HAV	6.50	5.50	≤2.49	1.00	≥4.01
HAV	7.90	6.50	≤2.49	1.40	≥5.41

n.e.p., no evaluation possible.
$TCID_{50}$/ml was calculated according to Spearman and Kärber [24].

Table 4
Virus inactivation experiments in the Lobator sd-2 (Lsd-2) with a control at 4 °C (values are given as $\log_{10}$)

Virus	Starting titer ($TCID_{50}$/ml)	Virus titre 94 min/4 °C ($TCID_{50}$/ml)	Virus titre 94 min/Lsd-2 ($TCID_{50}$/ml)	Reduction factor 94 min/4 °C ($TCID_{50}$/ml)	Reduction factor 94 min/Lsd-2 ($TCID_{50}$/ml)
BPV	5.75	6.00	≤1.49	None	≥4.26
HIV-2	7.00	5.75	≤1.49	1.25	≥5.51
PRV	8.50	7.50	≤1.49	1.00	≥7.01
PV-1	8.00	8.00	≤1.49	None	≥6.51
BVDV	6.00	6.25	≤1.49	None	≥4.51
HAV	8.00	7.49	≤1.49	0.51	≥6.51

The level of virus detection could be calculated as low as ≤1.49 $TCID_{50}$.

Considering the results obtained in the three independent experiments, it can be concluded that the incubation temperature of the controls (RT vs. 4 °C) has a significant effect on the spontaneous loss of infectivity. Furthermore, toxic effects observed with individual bone preparations hamper the determination of the reduction factor.

4. Discussion

To lower the risk of transmitting an infection via an allogeneic bone transplant, national and international recommendations and guidelines have been published over the past 10 years [8,17–21]. Criteria for the selection of bone donors and testing of the donors for markers of infection were recommended. To enhance the safety of the transplants, inactivation procedures like heat or chemical treatment and irradiation were introduced.

There are several reasons for the widespread application of the Lobator sd-2 system for the preparation of femoral head transplants by heat treatment. Femoral heads can be prepared from individual bones for tissue replacement in various orthopoedic and traumatological operations. Furthermore, pathogens transmissible by bone tissue are heat sensitive. Heat treatment as used in the Lobator system can inactivate microbial pathogens while retaining osteoinductivity, structure and stability of the bone [10]. The latter is affected by treatment with heat at ≥100 °C (autoclaving) or by pasteurization [25]. Chemical inactivation procedures are afflicted by insufficient permeability of the tissue and by problems in removing chemicals from the tissue after inactivation [15]. According to reports by several authors, irradiation largely destroys the osteoinductive properties of transplants, especially when high doses are used [12].

Heat treatment of femoral head grafts with the previous model, Lobator sd-1, has been examined in detail [9,22]. Femoral heads of different size and density served as the basis for the application of the treatment which took place in a water bath heated up within 30 min to 80 °C. The necessary exposure time to achieve a temperature of 80 °C over at least 10 min was determined and reached in the core even of large femoral

heads. Recent findings by CLB (personal communication) regarding BVDV and canine parvovirus (CPV) inactivation led to the suggestion to increase the effective temperature/time function (82.5 °C/15 min).

We provide evidence that in the Lobator sd-2 system the necessary parameters (82.5 °C, 15 min) for the inactivation of a variety of relevant and model viruses are reproducibly achieved in decartilaged femoral heads with a diameter $\leq$56 mm. When the diameter is $\geq$60 mm, the inactivation parameters are not reproducibly guaranteed. As expected, the polypropylene tube used in the virus model decreased the height and duration of the maximal temperatures and accordingly on the total heat quantity compared to production conditions. But in all femoral heads with a diameter between 51 and 58 mm the required temperature and holding time were reached. Therefore reduction factors determined in the virus model are not only transferable to production conditions of thermal disinfection of femoral heads, but represent a 'worst case'.

HIV is considered relevant for the safety of bone transplantation and was classified as weakly resistant to physicochemical treatment. It is well known that HIV is heat labile and is rapidly inactivated at $\geq$60 °C [22,26]. Validation of the Lobator sd-1 system regarding a sufficient inactivation of HIV was published previously [22] and confirmed in the present study.

It is well known that the virus species and the kind of environment (dry or moist heat, protein content) influence the heat resistance of viruses [27]. In suspension experiments at 65 °C over 15 min Lelie et al. [28] demonstrated a complete inactivation of vaccinia virus (Poxviridae), encephalomyocarditis virus (Picornaviridae), sindbis virus (Togaviridae), mouse hepatitis B virus (Coronaviridae), influenza virus (Orthomyxoviridae), vesicular stomatitis virus (Rhabdoviridae) and cytomegalovirus (Herpesviridae). A significant influence of the environment was demonstrated for parvoviruses. At a temperature of 80 °C, 15 min was required for the inactivation of BPV in drinking water, 27 min in double-distilled water and 9 min in cell culture medium. Wigand et al. [29] showed that heat resistance is increased by a factor of 3 when the virus in cell culture medium is diluted with double-distilled water (1:100). Under the influence of dry heat at 100 °C BPV shows exceptionally high resistance. Virus suspended in plasma shows higher heat resistance than virus suspended in double-distilled water [30]. Therefore direct contact of the viruses investigated with the centre of the femoral head was essential for validating the inactivation process.

HBV and HCV remain problematic viruses in the context of femoral head transplantation (as well as in the context of blood transfusion and organ transplantation) [31–33]. Both viruses cannot be used in in vitro cell culture systems and only to a limited extent in animal experiments [17,32]. For these reasons BVDV is considered as a model for HCV and parvoviruses as a model for highly heat-resistant viruses. In another model system for HCV Charm et al. [26] have shown that yellow fever virus as a representative of the Flaviviridae was completely inactivated at 60 °C after 5 min.

For two essential reasons human HBV cannot be used in validation studies of the Lobator sd-2 system: (1) at present, no generally suitable cell culture system is available, and duck hepatitis B virus (DHBV) which was used as a model for HBV in other inactivation studies [34] grows only in primary duck hepatocytes which were regarded as unsuitable for investigating heat-treated bone tissue. Furthermore, the German Advisory Committee Blood has stated that so far there is no suitable model virus for human HBV and that therefore such investigations cannot be made a requirement [32]. (2) After inactivation in the Lobator sd-2 the homogenized and centrifuged virus suspension contains remaining fat and bone tissue which inhibit an amplification of nucleic acids via PCR (inhibitors, disturbing factors). Therefore, PCR is not suitable for an assessment of the inactivation procedure under routine conditions and could only serve as an additional criterion of an efficacy assessment [35].

There are arguments in favour of using parvoviruses in validating thermal disinfection procedures regarding their efficacy for heat-resistant viruses [27]. The results by Bräuniger et al. [30] demonstrate that at 60 °C BPV shows heat resistance at least similar to HBV. Therefore in the assessment of heat resistance BPV can be used as a model virus for HBV in determining the efficacy of thermal disinfection procedures. In our investigations BPV was completely inactivated, i.e. by a factor of $\geq$4 $\log_{10}$. Borovec et al. [36] recommend PRV as a model for validating the inactivation of HBV. Our investigations also showed a complete inactivation of PRV as well as a sufficient titre reduction. In addition, the non-enveloped viruses investigated (HAV, PV-1) were completely inactivated in the Lobator sd-2 system, and by $\geq$4 $\log_{10}$ steps.

In conclusion, we can state that a sufficient titre reduction (4 $\log_{10}$ steps) of clinically relevant viruses is achieved in the Lobator sd-2 thermal disinfection system. In consideration of physiological fluctuations in morphology and fat content, a transversal diameter of the femoral head, of $\leq$56 mm, to be treated is recommended. In addition, the criteria for selecting donors in the guidelines for managing bone banks by the Bundesärztekammer should be observed and the required serological tests should be done as well. In case femoral heads from multi-organ donors are used, additional tests should be mandatory regarding HIV, HBV and HCV genome via nucleic acid amplification techniques (e.g. PCR).

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Design and management of an orthopaedic bone bank in the Netherlands

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Abstract The design and management of an orthopaedic bone bank is a complex process in which medical organisation and legislation intertwine. Neither in the Netherlands, nor in any other European country, there are official guidelines for the organisation and management of an orthopaedic bone bank. In the Netherlands, the recently modified 'law of security and quality for using human materials' (WVKL) dictates requirements for technical and organisational aspects for the use of human tissue and cells. The bone bank procedures include a thorough questionnaire for donor selection, extensive serological, bacteriological and histopathological examination, as well as standard procedures for registration, processing, preservation, storage and distribution of bone allografts. This article describes the organisation of an accredited bone bank and can be used as a proposition for an official guideline or can be useful as an example for other orthopaedic bone banks in Europe.

Keywords Orthopaedic bone bank · Legislation · Organization

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Introduction

For reconstruction of bone defects, donor bone from orthopaedic bone banks is often necessary. Bone grafts are used in bone defects that arise from trauma (Friedlaender 1987), infection, resection of bone tumours (Mankin et al. 1996) or it is used in spinal fusion (Raizman et al. 2009) and as impaction grafting in revision of total joint arthroplasty (Slooff et al. 1996).

Autologous bone is preferred because of its osteoconductive and osteoinducive activity, but it is often not sufficiently available and it repeatedly involves donor site morbidity (Summers and Eisenstein 1989). Allogenic bone exclusively has osteoconductive activity; it serves as a frame against which newly formed bone gets deposited (Elves and Pratt 1975; Urist 1953). Allogenic bone is provided by an orthopaedic bone bank.

In Leiden, the Netherlands, the Dutch Bone Bank Foundation (NBF) was founded in 1988 (Veen et al. 1990). In this central bone bank, bone- and tendon transplant material of deceased donor patients is stored (Veen et al. 1991). When needed, hospitals may order such material from the NBF.

A number of hospitals manage their own bone banks, such as the VU university medical center in Amsterdam, where an orthopaedic bone bank has been established. This VUmc orthopaedic bone bank contains only femoral heads of suitable patients who underwent total hip replacement surgery. The

advantage of possessing a bone bank is that the hospital always has its own supply of donor bone material; this may also be a financially viable strategy for hospitals carrying out many procedures for which donor bone material is required.

Up to now, nationally recognized guidelines for maintenance and management of bone banks do not yet exist in the Netherlands. In this paper we describe the VUmc orthopaedic bone bank procedure, which recently gained official approval and recognition and could serve as a potential outline for other hospitals.

Bone bank procedure

From October 2008, the Ministry of Health, Welfare and Sport (VWS) officially recognized the orthopaedic bone bank of the VUmc (Inspectie voor de Gezondheidszorg 2008). A biannual inspection is performed by the Health Care Inspectorate (IGZ) as a requirement to maintain this recognition. The bone bank procedure has to meet the requirements of the adjusted 'law of security and quality for using human materials' (Wet Veiligheid en Kwaliteit Lichaamsmateriaal 2003). This law became effective from mid-2007 in the Netherlands as a result of European guidelines 2004/23/EC and 2006/86/EC. These guidelines state the technical requirements for coding, processing, preserving, storing, and distributing of human tissue and cells. Human tissue should be traceable, and serious side effects and incidents with human tissue and cells should be reported.

The procedure of our orthopaedic bone bank is based on guidelines of The American Association of Tissue Banks (AATB), and the criteria of the Council for Blood Transfusion of the Netherlands Red Cross (Richtlijn Bloedtransfusie 2004), together with the recently merged Netherlands Bone Bank Foundation (NBF) and Bio Implant Services (BIS) (NBF-BIS Foundation 2010).

Previously, we followed the guidelines of the European Association of Musculoskeletal Transplantation. As a result of diverging European legislations, this organization has been discontinued as an European umbrella organization; currently only national associations prevail. To date, the Netherlands has not possessed such an association; consequently there is no national guideline with regards to maintenance and management of an orthopaedic bone bank.

The bone bank protocol

The bone bank procedure should be carefully described in an extensive protocol consisting of the following five components: organization, donor selection, documentation, storage and processing, and implementation. The HOD (Head of Department) of the Department of Orthopaedics and the bone bank administrator compose this protocol.

Organization

In an organization chart we describe the responsibilities of different stakeholders: the HOD of the Department of Orthopaedics, a bone bank administrator, a theatre nurse, a medical microbiologist, an anatomic pathologist, a clinical chemical analyst, a haematological laboratory technician, and a trainer. The HOD is the main responsible of the bone bank, whereas the bone bank administrator is responsible for the daily management. The knowledge and skills concerning surgical techniques and clinical hygiene are guaranteed by the orthopaedic surgeon and theatre nurse. The bone bank administrators' responsibilities include administration as well as storage and allocation of donor bone. Additionally, the administrator takes care of the maintenance and cleaning of the storage facilities (freezers, etc.), and verifies the registration forms of femoral heads meeting the requirements for storage in the bone bank. Both the bone bank administrator and the trainer are responsible for training of bone bank employees.

Apart from an orientation module for new employees, the training program consists of regular refresher courses for all members of the staff, in order to keep the knowledge of the procedures updated.

Donor selection

Preceding the hip replacement procedure, the attending orthopaedic surgeon requests the patient for his permission to store any removed tissue for donation. It concerns patients whose femoral head grafts will be retrieved in order to be replaced by a total hip prosthesis. Corticospongious bone tissue can not be sufficiently obtained in knee or shoulder arthroplasty; therefore patients undergoing such procedures cannot be taken into consideration for donor bone tissue donation.

The attending orthopaedic surgeon informs the patient both orally and in written. In case the patient grants permission he or she signs the consent forms, and fills out a standard survey (see Table 1). The orthopaedic surgeon now decides whether the patient is suitable for being a donor; he uses general and specific exclusion criteria (see Tables 2, 3). All criteria must be met; if not, exclusion necessarily follows. The orthopaedic surgeon examines the patient thoroughly: blood samples are collected to determine blood type, Rh-factor and erythrocyte sedimentation rate (ESR) (Tables 4, 5).

During surgery, bacterial culture swab samples from hip ligament are collected and a biopsy of corticospongious bone is sent off for histopathological analysis. Serological screening for infectious diseases is performed 6 months after surgery. Once all requirements are met (Tables 1, 2, 3, 4, 5), a femoral head can be released for donation:

- approval donor
- signed consent forms of donor
- completed survey; all questions should yield a negative answer
- preoperative ESR rates within criteria
- no abnormal bacteriological values in surgery derived tissue
- no abnormal histopathological structures in surgery derived tissue
- no abnormal serological values 6 months after surgery

Table 1 Questionnaire patient for orthopaedic bone donation

In the past 3 months, did you suffer any infection? If so, what infection?
In the past 3 months, did you have any vaccination or inoculation, or have you been injected with narcotic drugs?
In the past 6 months, did you have a malaria attack or did you use anti-malarial medication?
Have you ever been infected with a sexually transmitted disease?
Have you ever been diagnosed with jaundice or liver illness?
In the past 6 months, have you been in contact with patients diagnosed with jaundice/hepatitis?
In the past 6 months, have you been in contact with patients diagnosed with AIDS, or individuals at risk to AIDS? If yes, how and when?
Have you ever been tested for HIV/AIDS?
Have you had homosexual intercourse after 1977? (males only)
Have you emigrated after 1977? If so, to what country?
Are you diagnosed with haemophilia? If yes, are you using anticoagulants?
Are you a sexual partner of an individual for which any of the abovementioned questions can be answered with 'yes'?
Have you been actively involved in prostitution after 1977, or have you been a sexual partner of a person involved in prostitution in the past 6 months?
Have you ever been diagnosed with a haematological disease or any malignant disorder?
Have you ever been treated for diabetes mellitus?
Have you ever been treated for chronic brain- or neurological diseases?
Have you ever received radiation therapy?
Have you ever been diagnosed with rheumatoid arthritis?
Have you ever been diagnosed with tuberculosis?
Have you ever been diagnosed with any disease, other than the abovementioned?
Have you ever received hormonal treatment?
Do you use any prescribed medication?
Have you ever used any narcotic drugs?
Have you recently been exposed to hazardous or toxic materials? If yes, please specify.
What is your alcohol consumption per week?
Have you recently been in surgery? If so, when? Did you receive blood from a blood transfusion?
In the past 14 days, have you been traveling through or staying in a region exposed to a SARS epidemic, or have you been in contact with patients infected with SARS?
In the past 6 months, have you tattooed yourself or did you get a piercing?

Table 2 General exclusion criteria

No permission from patient
Under aged donor (<18 years)
Active or recent systemic infection/sepsis
Active infection of transplantation tissue (especially coxitis/osteomyelitis)
Previously infected with tuberculosis
Active "slow-virus" infection or anamnesis in the past
Anamnesis of previous infection with hepatitis B or C, AIDS or AIDS related complex, or tested positive for HIV
Active or past syphilis infection
Recent (<4 weeks) vaccination with live vaccine (measles, yellow fever, mumps, polio, oral typhoid, rubella)
Rheumatoid arthritis
Diffuse connective tissue disorders/autoimmune diseases
Metabolic disorders
Existing insulin dependent diabetes mellitus
Treatment with growth hormones
Chronic medication (especially corticosteroids)
Recent exposure to toxic substances
Malignancies
Donor location has been exposed to radiation
Chronic neurological disorders
Dementia
Language barrier or when patient does not understand the information for any reason (e.g. psychiatric patients)

Table 3 Specific exclusion criteria

A clinically proven HIV infection
Men having homosexual intercourse after 1977
Intravenous medication/narcotics use, currently or in the past
Immigrants (after 1977) from countries of which it is known that heterosexual intercourse is an important factor for HIV transmission
Haemophilic patients administered with clotting factors concentrate
Sexual partners from abovementioned individuals
Men and women active in prostitution since 1977, and individuals being their partner in the past 6 months
Individuals who recently (past 6 months) placed a tattoo or piercing
Individuals who have had a blood transfusion before 1980
Individuals who have had a blood transfusion outside Europe or North America
Individuals who stayed in a SARS epidemic area or individuals who had face-to-face contact with a SARS patient

Documentation

Accurate documentation and coding are a necessity for a well functioning bone bank. A unique registration code is allocated to each femoral head. Only the bone bank administrator is able to trace the donor based on this code. Of every registered femoral head, a file, containing the consent forms and results of ESR, bacteriological and histopathological examination, is kept updated. Other relevant data, such as the size of the femoral head and the allocation date are also documented and stored in this file. When the file is completed (which takes at least 6 months due to the serological examination), and no abnormalities are recorded, both bone bank administrator and the responsible orthopaedic surgeon sign the forms. The femoral head is now available for transplantation. In case a file cannot be completed in full, or any

Table 4 Haematological examination before surgery

Blood type and rhesus factor
Erythrocyte sedimentation rate (ESR), age and sex dependant
Normal values
Male
<50 years: 0–15
>50 years: 0–20
Female
<50 years: 0–20
>50 years: 0–30

Table 5 Serological examination 6 months postoperative

Viral (hepatitis)
Hepatitis B antigen
Hepatitis B antibody
Hepatitis C antibody
Viral nucleic acid
Viral (additional)
HIV
HTLV
Bacterial syphilis
TPHA

abnormal values are recorded, the femoral head will be destroyed according to hospitals' protocol.

Storage and processing

The femoral head is surgically removed under sterilized conditions. The ligament and synovial tissue are cultured on aerobic and anaerobic bacteria. In order to exclude malignancies, auto-immune processes, or infections, a biopsy of 1 cm^3 corticospongious bone and ligament is collected for histopathological examination. After determining its size, the femoral head is wrapped in a sterile plastic bag and in three layers parcelled in sterile packing material, labelled and stored in the freezer within 30 min.

The freezer has a temperature of −80°C, and has a continuous temperature registration device installed. Should the temperature fall outside the acceptable range of −90 and −70°C, an alarm system gives off a warning signal to the Technical Service, guaranteeing a 24-h security against temperature-induced damage to the tissue. A nitrogen tank is fitted onto the freezer, as a backup cooling mechanism in case of mechanical breakdown of the freezer. In deep frozen condition, the allogenic bone tissue can be preserved for a maximum of 5 years. The temperature data is stored and managed by the bone bank administrator for a period of at least 5 years.

Allocation and implementation

If during surgery a surgeon decides to use a femoral head as an allograft, a femoral head from the freezer together with its documents are handed over to the orthopaedic surgeon and surgery team. The orthopaedic surgeon and theatre nurse verify the file and expiration date of the femoral head. The femoral head is thawed in physiological saline; after being defrosted the theatre nurse takes a bacterial culture swab.

The hospital or care institution warrant fulfilment of the traceability requirements, which implies storing the file of the femoral head and records of the receiving patient for 30 years post implantation.

Discussion

Macewen first describes the use of allogenic human bone tissue in 1881 (Macewen 1881). From that year onwards, the use of allogenic bone transplantation has been increasingly applied and is nowadays a standard orthopaedic procedure (Tomford et al. 1987). However, much has changed in the past decade: donor selection, clinical hygiene, storage and processing, allocation, implantation, and documentation are bound to strict rules.

Donor selection takes place by a thorough broad survey and supplementary physical examination. The survey should be regularly revised to comprise the latest knowledge and developments, and as a response to the spread of new infectious diseases. For instance, after the outbreak of the SARS epidemic in certain countries, a question was added to the survey; prospective donors were asked whether they had been in SARS-infected regions or whether they had been in contact with an infected person. It is not unthinkable that newly arising infectious diseases will be included in the survey and henceforth become exclusion criteria.

Laboratory examination is performed before surgery, including ESR determination. Elevated values are often encountered, often without clinical implications. However, the exclusion criteria are strictly enforced. Preoperatively, blood type and Rh-factor are determined. The Rh-factor only becomes an issue if the receiving patient is a young woman. Additionally, the donor is serologically examined, to exclude possible transmission of infections, such as HIV and hepatitis (Strong et al. 1996; Shutkin 1954; Patel and Trampuz 2004; Karcher 1997). The serological tests are carried out at least 6 months after surgery to avoid type II error (false negative): if the patient was infected at the time of surgery, the test will provide conclusive evidence of this. Normally, the donor will not be informed of the results, unless specifically requested by the donor.

In addition to existing national and international guidelines and procedures of the AATB, NBF-BIS, and former EAMST, histopathological examination is added to the current bone bank procedure. Previous studies have found pathological abnormalities in 8%, and B-cell lymphomas in 2,2% of donor femoral heads (Palmer et al. 1999; Zwitser et al. 2009; Sugihara et al. 1999). Though transmission of malign cells following transplantation has never been shown, femoral heads with histopathological abnormalities are excluded from allogenic bone transplantation.

Apart from an orthopaedic bone bank, many hospitals often have more organ banks, such as a haematological bone marrow bank for stem cell transplantation or the IVF laboratory of the department of Gynaecology and Obstetrics. Therefore, in every hospital a central system for tissue vigilance should be established and all departments that require cells and/or tissue for transplantation purposes should participate. Thus, highest cell and tissue security levels within the hospital are warranted by controlling the complete transplantation chain from donation to transplantation, including appropriate reporting and follow-up of incidents and side effects.

Conclusions

The design and management of an orthopaedic bone bank as an organ bank is a very complex process in which aspects of medical organization and legislation intertwine. In this paper we describe our bone bank procedure, which is approved and recognized by the Ministry of Health, Welfare and Sport (VWS) of the Netherlands. This procedure could serve as a provisional Dutch protocol for the setup and maintenance of other orthopaedic bone banks.

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ORIGINAL ARTICLE

BONE ALLOGRAFT BANKING IN SOUTH AUSTRALIA

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The South Australian Bone Bank has expanded to meet an increased demand for allograft bone. During a 5 year period from 1988 to 1992, 2361 allografts were harvested from 2146 living donors and 30 cadaveric donors. The allografts were screened by contemporary banking techniques which include a social history, donor serum tests for HIV-1, HIV-2, hepatitis B and C, syphilis serology, graft microbiology and histology. Grafts were irradiated with 25 kGy.

The majority of grafts were used for arthroplasty or spinal surgery and 99 were used for tumour reconstruction. Of the donated grafts 336 were rejected by the bank. One donor was HIV-positive and two had false positive screens. There were seven donors with positive serology for hepatitis B, eight for hepatitis C and nine for syphilis. Twenty-seven grafts had positive cultures.

Bone transplantation is the most frequent non-haematogenous allograft in South Australia and probably nationally. The low incidence of infectious viral disease in the donor population combined with an aggressive discard policy has ensured relative safety of the grafts. The frequency of graft rejection was similar to other bone banks but the incidence of HIV was lower.

Key words: allograft, bone graft, bone banking, HIV.

INTRODUCTION

Transplantation of bone allografts has an established role in orthopaedic surgery and where there are centralized tissue banks it has been shown to be the most frequently transplanted allograft excluding blood and blood products. There were an estimated 200 000 allograft recipients in the United States in 1989[1] and 6000 allografts were performed by 45% of the clinical hospitals during the federal survey of German Surgical Clinics in the same year.[2] In Australia it has been suggested that the most frequently transplanted tissue is cornea;[3] however, a review of the South Australian Bone Bank in this study suggests that bone is the most frequently transplanted tissue.

Since the initial report of the South Australian Bone Bank[4] it has been expanded and undergone organizational changes to have it established as an institutional bone bank servicing the public and private sector hospitals of the State of South Australia. Bone harvested during a 5 year period since these organizational changes is the subject of this review which includes the years 1988–92.

An audit of the South Australian musculoskeletal bank was undertaken to determine the frequency and patterns of bone allografting in that State. Of particular interest were the demographic data of the donor population and allograft bones that were discarded to determine the cause and incidence of bone being rejected.

METHODS

Bone donations are received from two sources. Femoral head specimens are obtained at the time of total hip replacement or hip hemiarthroplasty for fracture. Long bones and pelvic bones are harvested from cadaveric organ donors as part of the State wide general organ transplantation programme.

Femoral head donors are required to sign a declaration stating the absence of risk factors to potentially infectious disease transmission as outlined in a pre-donation proforma modelled on the American Association of Tissue Banks.[5] The donors are interviewed by the donating surgeon who completes the proforma listing exclusions directed at the possibility of transmissible viral diseases, bacterial infection, tumours and diseases of unknown aetiology including connective tissue diseases, diabetes and Paget's disease. The bone bank co-ordinator may assist with donor screening and will exclude any donors with an incomplete or unsuitable history.

Pre-donation screening tests are performed at the time of completion of the risk declaration form. The screening tests may precede surgery by several weeks and there is the potential for bone to be excluded before it is sent to the bone bank if there is an abnormal result. The following screening tests are performed; HIV-1 and HIV-2 antibody (recombinant HIV-1/HIV-2 third generation, Abbott, Chicago, IL, USA), hepatitis B antigen (Auszyme, Abbott), hepatitis C antibody (HCV EIA 3.0, Abbott), syphilis serology (Centocore EIA/G, Centocore, USA). ABO and Rhesus blood groups were recorded but donor and recipient matching was infrequent.

Cadaveric organ donors are screened by proxy via relatives and the treating physicians who are usually from an intensive care environment. The co-ordinator of the South Australian Organ Donation Programme assists with counselling relatives and assessment of donor risk factors. Pre-donation screens are the same as for femoral head donors with the addition of blood cultures.

All specimens are removed under strictly aseptic conditions, femoral head donors and most long bone donors are harvested in the operating room, 27% of long bone donors were harvested in the autopsy room. Specimens are triple wrapped in sterile

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plastic bags or containers or both and stored at −70°C. Antibiotics used during hip arthroplasty surgery are withheld until bacteriological swabs are taken. Bacteriological culture swabs are taken at the time of harvesting and include the cut bone surface and synovial fluid. Swabs are incubated in Stuart's transport medium and subcultured onto blood agar and culture broth for a further seven days. A biopsy of the specimen is examined by a histopathologist.

During the time of this review all specimens were irradiated with 25 kGy of gamma irradiation (Cobalt 60 source) while maintained on dry ice.

All consecutive allograft specimens during this period were reviewed by analysis of donor and recipient demographic factors, donor proforma questionnaires and pre-donation screening tests.

RESULTS

Donations

During the period of review 2361 bone grafts were collected from 2176 patients (Fig. 1). There were 2146 living donors including 1856 femoral heads and 296 knee bones. Bone wedges from knee arthroplasty surgery were harvested when the demand for femoral head bone exceeded supply. Sixty-five per cent of femoral head allografts were received from metropolitan public hospitals and 35% from private hospitals or the Mount Gambier Regional Hospital. From 30 donors 215 long bones were harvested.

Donor demographical data are included in Table 1. There was an approximately equal sex distribution at each age range. The majority of living donors were aged greater than 60 years and most of the cadaveric donors were younger adults. Of the cadaveric donors 60% died from non-violent medical illness (Table 2).

Bone usage

The distribution of living and cadaveric allografts is included in Table 3. At the time of data collection all the living donor allografts had been used and 140 of the 215 cadaveric allografts had been used. The most frequent indication for bone allografting was arthroplasty surgery and spinal fusion. Femoral heads were used as structural grafts or milled bone chips and more than one femoral head was often used during the recipient procedure. Long bones used for arthroplasty surgery were most often utilized as proximal femoral allografts and one or two bones were required with the second being utilized as a strut graft.

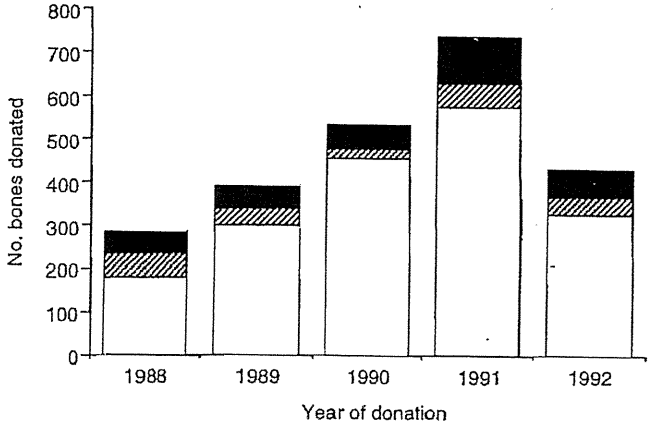

Fig. 1. South Australian Bone Bank donations 1988–92. (■), discarded bones; (▨), cadaveric donors; (□), living donors.

Table 1. Bone donor age distribution

Age	Living donors	Cadaveric donors
13–19		3
20–29	5	7
30–39	11	4
40–49	69	6
50–59	228	7
60+	1507	3
Unknown	4	
Total	1824	30

Table 2. Cadaveric donor cause of death

Cause of death	No. of donors	No. of bones
Intracranial haemorrhage	13	94
Cardiogenic	7	49
Vehicle accident	6	28
Suicide	3	36
Accidental shooting	1	8
Total	30	215

Table 3. Allograft bone recipient procedures

Recipient procedure	Living donors	Cadaveric donors
Arthroplasty	758	80
Spinal fusion	675	10
Tumour	66	33
Fracture/osteotomy	105	
Maxillofacial	27	
Other	47	17
Unspecified	52	
Total	1730	140

The bone bank was available to all surgeons but was most frequently utilized by orthopaedic surgeons. A smaller number of grafts were used by neurosurgeons and maxillofacial surgeons.

Discarded allografts

Three hundred and thirty-six allografts were discarded. Of these 77 were discarded because of a positive donor history such as a previous tumour or Paget's disease. Seventy-one bones were discarded because of incorrect handling procedures including incomplete donor history or screening tests, mishandling of the grafts (such as placing the graft in formalin) or an excessive delay in refrigeration after procurement. After collection 103 bones were rejected due to logistical errors mostly when the graft had been returned unused and allowed to thaw.

There were 85 exclusions because of positive patient or graft screens (Table 4). Biopsy exclusions were mostly non-specific such as excess numbers of inflammatory cells but also included an undiagnosed Paget's disease and an undiagnosed lymphoma.

'able 4. Bone grafts excluded with positive screening test

ositive result	Total
iopsy	31
ulture	27
enereal Disease Research Laboratory	9
epatitis C	8
epatitis B	7
IV test positive	2
IV true positive	1
otal	85

Culture positive results included 17 *Staphyloccocus epidermidis*, four *Staphyloccocus aureus*, two *streptococci*, two anaerobic diphtheroids, one Gram-negative and one Gram-positive acillus.

Three bone donations were rejected because of a positive IIV-antibody test result. One test result was confirmed positive y western blot. This patient had an elective total hip replacement and donated his femoral head without knowledge of his ntibody status which was determined as a result of bone bank creening for HIV antibody. The patient had unknowingly igned his consent form denying any risk factors; the proforma t that time did not include heterosexual contact with sex orkers from an area endemic to HIV (Thailand) and was the ause of infection in this case. Two other patients were rejected ecause of an initial HIV-positive test result, one was an incorect interim report from the laboratory, the second was an lderly woman with no risk factors who returned repeat intermediate test results and did not seroconvert on repeat testing at months.

DISCUSSION

ince the resurgence of interest in bone allografting in the 970s there have been well established reviews and guidelines escribing the methods of harvesting and storage of bone in one banks.[5–8] The United States bone collection and transplantation programmes are generally conducted on an institutional basis and community hospital based programmes emain the major source of bone banking in Australia and the Jnited Kingdom due to the limited resources required to establish these smaller banks.[9] The South Australian Bone Bank emains a hospital-based bank but since the first year of the ank's operation[4] there has been increased demand for allograft bone. The bone bank has expanded to meet this need nd now services 20 public and private hospitals within South ustralia.

During the 5 year period of this review 2361 allografts were ollected from 2176 donors. During the same period the South ustralian organ donation programme harvested 510 organ and issue allografts from 115 donors. During a 6 year period the ion's Eye Bank of South Australia collected corneas from 790 onors.[10] Nationally there were 3585 cornea transplants and 787 kidney transplants from 1986 to 1991.[3] National figures or bone allografts are not recorded but the current study sugests that the number of bone allograft procedures exceeds orneal grafting, which was previously believed to be the most requent tissue/organ allograft.

A feature of our bank has been its priority of graft safety articularly since the majority of grafts were used for elective surgery such as arthroplasty and spinal fusion. There is a low incidence of significant viral disease carriers in the donor community and the bank has an aggressive discard policy. Initially there was a high discard rate of 46% during the foundation year[4] but this has been decreased to 14% in the current review. The majority of grafts continue to be rejected as a result of handling and logistical errors such as incomplete documentation, inadequate screening tests or poor graft handling at procurement or unused returns.

As a result of positive screening tests 3.6% of grafts were rejected and the bacterial infection rate was 1.1%. The infection rate has fallen dramatically from the initial year when 17% of grafts had positive cultures. This decline is partly explained by the inclusion of approximately 5% of grafts which had a light growth of *S. epidermidis* in broth culture. Previous reports of positive cultures have varied from 2.2 to 22%.[9,11,12] The majority of banks perform microbiological screening of bone grafts but the significance of a positive culture varies with the philosophy of the tissue bank, some banks discard bone that is found to have bacterial contamination and others will accept known contamination and rely upon secondary sterilization.[12]

All grafts were irradiated with 25 000 Gy of gamma irradiation as recommended by the International Atomic Energy Agency[13] but this may not be sufficient to inactivate viruses such as HIV.[14,15]

Infectious viral diseases continue to be a major concern to allograft banks particularly when the donor population has a significant incidence of viral carriers. The American Red Cross Transplantation Services reported 0.46% hepatitis-B-surface-antigen-positive and 0.3% HIV-positive[9] which contrasts to the South Australian Bone Bank figures of 0.3% hepatitis-B-positive and 0.04% HIV-positive. The relative low incidence of HIV has been reported in other Australian blood and tissue banks. Forty six of 5.4 million Australian blood donations were HIV-positive[16] and the South Australian Lions Eye Bank identified two HIV-antibody-positive donors and three hepatitis-B-positive donors from 790 donors.[10]

The donor demographic data and medical history remain the most important factors to decrease the risk of donor to host virus transmission.[17] Femoral head donors tend to be of the age range least associated with HIV and hepatitis transmission and they offer the ability to obtain a more detailed medical history. The pre-donation proforma directs questions to the risk of HIV and hepatitis transmission, neurological slow virus transmission (for which there are no screening tests freely available), current or previous malignancies (although there have been no reports of malignancy arising from organ or tissue allografts) and other conditions of unproven aetiology such as connective tissue disorders, diabetes and Paget's disease.

Cadaveric donor bone may be less safe than bone from living donors because of the donor's age and mode of their death.[18] Only 9% of bone allografts in this series were from cadaveric donors but it may be an important source of bone in some American tissue banks who receive donors from major trauma including social violence such as gunshot and knife wounds.[19] Trauma patients presenting to trauma centres have a high incidence of HIV from 0.04 to 16%[20,21] and 1.3% was reported in an Australian series.[22] The majority of cadaveric donors in the current series died from non-violent medical illness.

Screening for HIV and hepatitis was introduced in 1985 and