Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biologicals 31 (2003) 75–82
BIOLOGICALS
www.elsevier.com/locate/biologicals

Инактивация вирусов в костных тканевых трансплантатах (головках бедренных костей) нагреванием при помощи Марбургской системы костного банка "

Axel Pruss[a*], Moujahed Kao[b], Thomas von Garrel[c], Lars Frommelt[d], Lutz Gürtler[e], Frank Benedix[a], Georg Pauli[b]

[a] *Institute for Transfusion Medicine (Tissue Bank), University Hospital Charité, CCM, Schumannstrasse 20/21, 10117 Berlin, Germany*
[b] *Robert Koch-Institut, Retrovirology, Berlin, Germany*
[c] *Clinic for Traumatology, Philipps University, Marburg, Germany*
[d] *Institute for Infectiology, Clinical Microbiology and Hospital Hygiene, ENDO-Clinic, Hamburg, Germany*
[e] *Friedrich Loeffler Institute for Medical Microbiology, Ernst Moritz Arndt University, Greifswald, Germany*
Received 10 September 2002; accepted 15 October 2002

Резюме

Несколько процедур инактивации вирусов, такие как термообработка, гамма-облучение и химическая стерилизация используются для повышения безопасности при трансплантации костной ткани. В этом исследовании мы представляем данные по инактивации вирусов посредством тепловой дезинфекции бедренных головок человека, используя Марбургскую систему костного банка"Lobator SD-2". Были исследованы три оболочечных вирусов (вирус иммунодефицита человека типа 2 [-2 ВИЧ], бычий вирус вирусной диареи в качестве модели для вируса гепатита С [ВГС], и вирус герпеса псевдобешенства), и три безоболочечных вирусов (вирус гепатита А, вирус полиомиелита и бычий парвовирус).

В модельной системе центральная часть головки бедра человека была контаминирована соответствующей бесклеточной суспензией вируса, посредством прямого контакта между вирусом и нативной костной тканью. Температура ядра в головки бедра во время процесса стерилизации была определена в дополнительных модельных экспериментах. Температуру 82,5 С, указанную изготовителем в качестве температуры эффективной инактивации вирусов, поддерживали в течение, по крайней мере 15 минут в очищенных от хряща бедренных головок с диаметром 56 мм. Термическая обработка с помощью Lobator SD-2 обеспечивала инактивацию всех вирусов в головках бедренных костей человека ниже предела обнаружения (по крайней мере, на коэффициент R4 log10).

Высокий уровень безопасности может быть достигнут путем совмещения тщательного отбора доноров и серологического определения соответствующих маркеров инфекций (anti-HIV-1/2, HBsAg, anti-HBcore, anti-HCV, TPHA) с термообработкой бедренной головы в Lobator SD-2. Для дальнейшего повышения вирусной безопасности при трупных трансплантаций кости, рекомендуется проводить проверку полиорганных доноров ДНК- тестированием (т.е. ПЦР) на ВИЧ-инфекцию, гепатиты В и С.

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

1. Введение

Несмотря на активизацию усилий по разработке альтернативных материалов и процедур, аллогенная трансплантация кости остается незаменимым инструментом для восстановления обширных костных дефектов. В частности трансплантация кости используется в хирургии для замены эндопротеза в тазобедренном суставе, при операциях на позвоночнике и реконструкции после обширных резекций опухоли.

В Германии ежегодно выполняется 71 000 аутологичных и 25 000 аллогенных трансплантаций костей ежегодно с дополнительной потребностью в примерно 18000 трансплантациях [1]. В США число аллогенных трансплантатов костного имплантировали исчисляется 300000 - 400000 в год [2].

В дополнение к клиническим или функциональным аспектам, риск передачи патогенов с помощью трансплантации вызывает большую озабоченность [3,4]. О передаче вирусов и патогенных микроорганизмов костной тканью сообщалось в источниках[5,6]. Тем не менее, отсутствие сообщений о передаче агентов, вызывающих ЦЭС как болезнь Крейтцфельда-Якоба при аллогенных трансплантациях кости, подразумевает, крайне низкий риск передачи TSE агентов с помощью костной ткани [7].

* Corresponding author. Tel.: +49-30-450-525161; fax: +49-30-450-525976
E-mail address: axel.pruss@charite.de (A. Pruss).

1045-1056/03/$30.00
doi:10.1016/S1045-1056(02)00095-7

В качестве исходного материала для производства костных трансплантатов используются кости конечностей, такие как головка бедренной кости и большой берцовой кости, позвонки и подвздошная
кость. Головки бедренных костей особенно хорошо подходят для коррекции неправильного положения, а также для индивидуальной адаптации трансплантатов при эндопротезировании, потому что они могут быть точно подобраны по размеру и форме, обеспечивая прочность соединения.

Для гарантии отсутствия патогена в ткани должны быть соблюдены специальные требования. В дополнение к их неизменной эффективности, центральным вопросом в оценке качества должна быть валидация дезинфекции или стерилизации тканей. В соответствии с руководящими принципами управления костными банками немецкий медицинской ассоциации, только подвергнутые иннактивации аллогенные кости или костные материалы, могут быть использованы для трансплантации в тех случаях, когда имеет место невозможность тестирование донора после достаточно долгого карантина [8].

В настоящее время в производстве трансплантатов костной ткани используются такие методы инактивации вирусов как термообработка [9,10], гамма-облучение [11,12], обработка надуксусной кислотой и этанолом [13-15]. В Германии распространенным методом является термообработка головки бедра в системе Lobator SD-2 (телос, Марбург, Германия). В отличие от других методов снижения патогенов, теплофизические дезинфекции сохраняет надлежащие биологические свойства кости [10,16]. Хотя предыдущие валидационные исследования показали соответствие процедуры требованиям национальных и международных стандартов [10,17-21], систематических исследований дезинфекции системой Lobator SD-2 было проведено не достаточно.

Целью этого исследования была проверка вирус-инактивации системой Lobator SD-2 контаминированных вирусом головок бедренных костей человека. Мы разработали модель системы для проверки процедуры инактивации, имитирую условия in vivo. Центр головки бедра человека был контаминирован высоким титром безклеточной вирусной суспензии, создавая прямой контакт вируса и кости. Это представляет особый интерес, так как в предыдущих исследованиях ПЦР пробирки, содержащие вирусную суспензию были помещены в центр головки бедренной кости. В такой модели системы не представляется возможным определить влияние компонентов крови и костей на устойчивость вируса [22].

2. Материалы и методы

2.1. Вирусы

Выбор вирусов производился в соответствии с рекомендациями Института Пауля Эрлиха и Федерального института лекарственных средств и изделий медицинского назначения (Paul-Ehrlich-Institut и Bundesinstitut fu¨r Arzneimittel und Medizinprodukte) [17]. Использовались следующие вирусы:

2.1.1. Оболочечные вирусы

Вирус иммунодефицита человека типа 2 (ВИЧ-2), ssRNA, Retroviridae, изолированнQҲ SBL6669 - перевиваемые суспензионные клеточные линии Molt 4 clone 8 cells. Этот вирус является моделью для ВИЧ-1. Он классифицируется как имеющий низкую резистентность к физико-химической обработки и инактивируется при 60 (С [19,20,22].

Hdл҃Gҥ ҒQƐqҲҩ ғdл҃GҥҀҁҩ ҳdыл҃Ҳd &@TBT'* qqPL ?* Dj_tgtgpgb_c* ɛ ҥыҽҽ Se 37-ПыҀd * ҩыҩ ҽҁҳҲҼd ғdл҃Gҥы ҳҲЉыҥdҥы ҳҼ A ғdл҃Gҥы &FAT'* +@TBT ғQл҃ыЈdғыҼd ғ WҽҒл҃dҁҀыҼqҀҁҩ ҥҲҼ ƐҲҩ ҩҼҲҥҩҲ, @TBT d FAT ҁҒы Љл҃dҀыҳҼҲӿыҥ ҩ ҥҲҽҲҩҥҥғG Dj_tgtgpgb_c d dҽҲwҥ ҥҁЉҁҥҥығdҽQҲ ɢddddҁ+ҍdҽdƐҲҥҩdҲ ҥғҁҩҥҥғы &dыЉл҃ҁҥ ӯҲҀҥл҃ыҼqҀҁҩ ҼыҒҁл҃ыҥҁл҃dd ШdҳҲл҃ҼыҀҳҁғ* ҥҼGӿҒы ЉҲл҃ҲҼdғыҀd ҩл҃ҁғd ПҲЉыл҃ҥыҽҲҀҥы ҩҼdҀdƐҲҥҩҁҩ Hdл҃ҁ+ УҽҽGҀҁҼҁҳd YAJ@[d Pcd, Y/7[',

Hdл҃Gҥ Nqcsbmp_`gcq &NPT9 ғdл҃Gҥ ҒҁҼҲdҀd ЛGҲҥҩd ҥғdҀҲҩ'* BQ ПШХ* ҳҲл҃ЉҲҥғdл҃Gҥҁғ* ɛ ҥыҽҽ Мыл҃ҥы ғQл҃ыЈҲҀҀQҍ ғ ҀҁЉҩҁғQҍ ҩҼҲҥҩыҍ ҼҲҳҩdҍ, ЪЫН dҥЉҁҼqdGҲҥҥ ғ ҩыƐҲҥҥғҲ ҽҁҳҲҼd ҳҼ ƐҲҼҁғҲҩы ғdл҃Gҥҁғ ҳҲл҃ЉҲҥы, Ґҥҁ ҥƐdҥыҲҥҥ GҽҲл҃ҲҀҀҁ Gҥҥҁҩ ƐdғQ ҩ ҥҲл҃ҽdƐҲҥҩҁҩ ҁҒл҃ыҒҁҥҩҲ Y/7[,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.

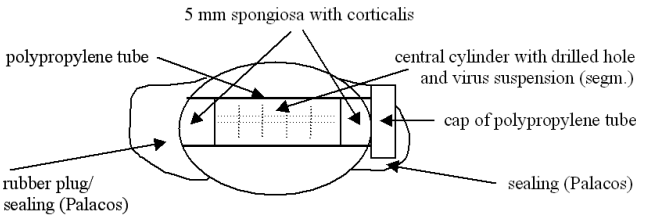

Fig. 1. Schematic drawing of the femoral head prepared for the validation study.

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.

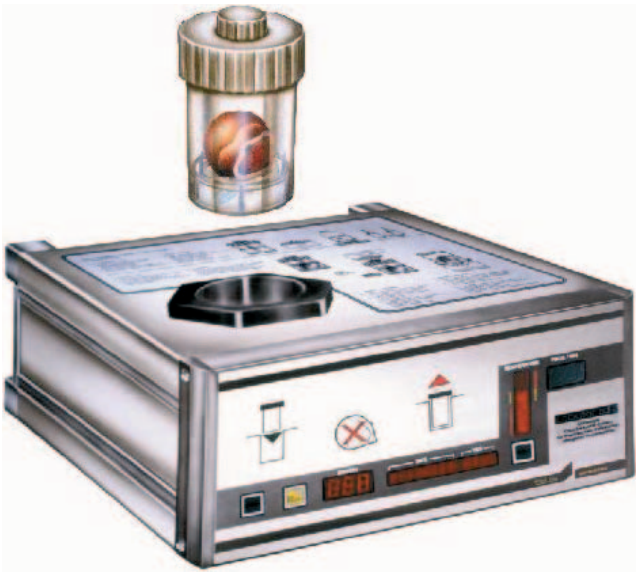

Fig. 2. Marburg bone bank system Lobator sd-2.

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:

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

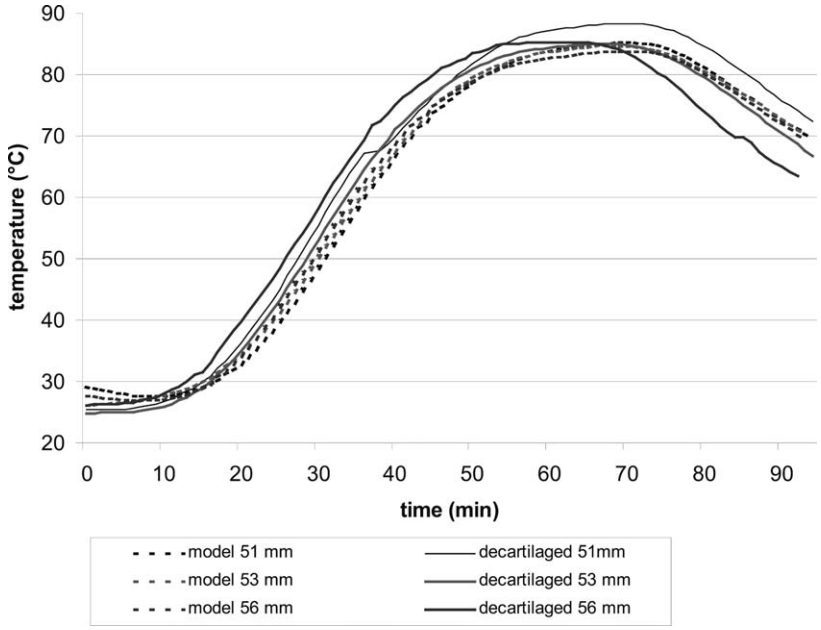

Fig. 3. Temperature kinetics in femoral heads.

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	$\leq$2.49	3.75	$\geq$6.00
BPV	8.25	5.74	$\leq$2.49	2.51	$\geq$5.76
BPV	7.49	3.74	$\leq$1.49	3.75	$\geq$6.00
PRV	8.00	4.49	$\leq$2.49	3.51	$\geq$5.51
PRV	8.37	6.25	$\leq$1.49	2.12	$\geq$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	$\leq$1.49	$\leq$1.49	$\geq$4.06	$\geq$4.06
HIV-2	5.00	$\leq$1.49	$\leq$1.49	$\geq$3.51	$\geq$3.51
BVDV	6.74	5.49	$\leq$2.49	1.25	$\geq$4.25
BVDV	6.62	5.25	$\leq$2.49	1.37	$\geq$4.13
HAV	7.40	4.88	$\leq$1.49	2.52	$\geq$5.91
HAV	6.50	5.50	$\leq$2.49	1.00	$\geq$4.01
HAV	7.90	6.50	$\leq$2.49	1.40	$\geq$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	$\leq$1.49	None	$\geq$4.26
HIV-2	7.00	5.75	$\leq$1.49	1.25	$\geq$5.51
PRV	8.50	7.50	$\leq$1.49	1.00	$\geq$7.01
PV-1	8.00	8.00	$\leq$1.49	None	$\geq$6.51
BVDV	6.00	6.25	$\leq$1.49	None	$\geq$4.51
HAV	8.00	7.49	$\leq$1.49	0.51	$\geq$6.51

The level of virus detection could be calculated as low as $\leq$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 $\geq$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).

Acknowledgements

The authors thank Dr Katharina Rokos, Mr Sven Schurig and Mr Frank Schweiger for excellent support during the experimental work.

References

[1] Jerosch J. Knochenbanken in der BRD. Ergebnisse einer Befragung. Unfallchirurg 1990;93:334–8.
[2] von Garrel T, Gotzen L. Allogeneic bone transplantation and bone banking. Unfallchirurg 1998;101:713–27.
[3] Kakaiya R, Miller WV, Gudino M. Tissue transplant-transmitted infections. Transfusion 1991;31(3):277–84.
[4] Wilmes E, Gürtler L, Wolow H. Zur Übertragung von HIV-Infektionen durch allogene Transplantate. Laryngol Rhinol Otol 1987;66:332–4.
[5] Habal MB, Reddi AH. Bone grafts and bone substitutes. Philadelphia: W.B. Saunders, 1992.
[6] CDC. Transmission of HIV through bone transplantation: case report and public health recommendation. MMWR 1988; 37:597–9.
[7] EMEA/CPMP. Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products. EMEA/410/01 rev 1, London; 31 May 2001.
[8] Wissenschaftlicher Beirat der Bundesärztekammer. Richtlinien zum Führen einer Knochenbank. Dtsch Ärzteblatt 2001;98(15): A1011.
[9] Hofmann C, Garrel T, von Gotzen L. Knochenbankmanagement bei Verwendung eines thermischen Desinfektionssystems (Lobator SD-1). Unfallchirurg 1996;99:498–508.
[10] Knaepler H, von Garrel T, Gotzen L. Untersuchungen zur Desinfektion und Sterilisation allogener Knochentransplantate. Hefte Z Unfallchir 1994;235:1–101.
[11] Smith RA, Ingels J, Lochemes JJ, Dutkowsky JP, Pifer LL. Gamma irradiation of HIV-1. J Orthop Res 2001;19:815–9.
[12] Pruss A, Kao M, Gohs U, Koscielny JK, von Versen R, Pauli G. Effect of gamma irradiation on human cortical bone transplants contaminated with enveloped and non-enveloped viruses. Biologicals 2002;30(2):125–33.
[13] Pruss A, Kao M, Kiesewetter H, von Versen R, Pauli G. Virus safety of avital bone tissue transplants: evaluation of sterilization steps of spongiosa cuboids using a peracetic acid–methanol mixture. Biologicals 1999;27:195–201.
[14] von Versen R, Heider H, Kleemann I, Starke R. Chemische Sterilisation biologischer Implantate mit einer Kombinationsmethode. In: Pesch H-J, Stöß H, Kummer B, editors. Osteologie aktuell. Philadelphia: Bern, 1992;vol. 2 (Suppl):380–6.
[15] Pruss A, Baumann B, Seibold M, Kao M, Tintelnot K, von Versen R et al. Validation of the sterilization procedure of allogeneic avital bone transplants using peracetic acid–ethanol. Biologicals 2001;29:59–66.
[16] Munting E, Wilmart JF, Wijne A, Hennebert P, Delloye C. Effect of sterilization on osteoinduction. Comparison of five methods in demineralized rat bone. Acta Orthop Scand 1988;59(1):34–8.
[17] Bekanntmachung des Paul-Ehrlich-Institutes und des Bundesinstitutes für Arzneimittel und Medizinprodukte. Anforderungen an Validierungsstudien zum Nachweis der Virussicherheit von Arzneimitteln aus menschlichem Blut oder Plasma. Bundesanzeiger vom. vol. 84; (04.05.1994). p. 4742–4.
[18] Europäische Norm EN 1040. Chemische Desinfektionsmittel und Antiseptika, Bakterizide Wirkung (Basistest) Prüfverfahren und Anforderungen. Brüssel: CEN (Europäisches Komitee für Normung); 1997.
[19] CPMP. Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. CPMP/BWP/268/95, final version. London; February 29, 1996.
[20] CEN. Sterilization of medical devices utilizing tissues—validation of the inactivation of viruses and other transmissible agents. 316/WG3/N-02; July 20, 1994.
[21] Bundesinstitut für Arzneimittel und Medizinprodukte. Bekanntmachung zu allogenen Gewebetransplantaten. Bundesanzeiger 1996;75:4670.
[22] von Garrel T, Knaepler H, Gürtler L. Untersuchungen zur Inaktivierung von HIV-1 in humanen Femurköpfen durch Verwendung eines thermischen Desinfektionssystems Lobator SD-1. Unfallchirurg 1997;100:375–81.
[23] Scheidler A, Rokos K, Reuter T, Ebermann R, Pauli G. Inactivation of viruses by beta-propiolactone in human cryo-poor plasma and IgG concentrates. Biologicals 1998;26:135–44.
[24] Spearman A, Kärber G. Cavalli-Sforza L, editor. Biometrie. Grundzüge biologisch-medizinischer Statistik. Stuttgart: Gustav Fischer, 1974;171–3.
[25] Herr G, Schmid U, Holz G, Reutter K, Schnettler R. Einfluss verschiedener Desinfektions- und Sterilisationsverfahren auf die biologische Aktivität und Struktur von Knochengewebe. In: Schnettler R, Markgraf E, editors. Knochenersatzmaterialien und Wachstumsfaktoren. Stuttgart: Georg Thieme, 1997;84–7.
[26] Charm E, Landau S, Williams B, Horowitz B, Prince AM, Pascual D. High-temperature short-time heat inactivation of HIV and other viruses in human blood plasma. Vox Sang 1992; 62:12–20.
[27] Bräuniger S, Peters J, Borchers U, Kao M. Further studies on thermal resistance of bovine parvovirus against moist and dry heat. Int J Hyg Environ Health 2000;203(1):71–5.
[28] Lelie PN, Reesink HW, Lucas CJ. Inactivation of 12 viruses by heating steps applied during manufacture of a hepatitis B vaccine. J Med Virol 1987;23:297–301.
[29] Wigand R, Bachmann M, Brandner G. Stabilization of isomeric DNA viruses against thermoinactivation by lowered ionic strength. Arch Virol 1981;69:61–9.
[30] Bräuniger S, Fischer I, Peters J. Zur Temperaturstabilität des bovinen Parvovirus. Zbl Hyg 1994;196:270–8.
[31] Roggendorf M. Bedeutung der HCV-Infektion für die Posttransfusionelle Hepatitis (Significance of HCV infection for post-transfusion hepatitis). Infus Ther Transfus Med 1994;1(Suppl):12–8.
[32] Burger R, Gerlich W, Gürtler L, Heiden M, Hitzler W, Jansen B et al. Hepatitis B virus. Infus Ther Transfus Med 2000;27:226–34.
[33] Eggen BM, Nordbo SA. Transmission of HCV by organ transplantation. N Engl J Med 1992;326(6) discussion 412–3.
[34] Murray SM, Freiman JS, Vickery K, Lim D, Cossart YE, Whiteley RK. Duck hepatitis B virus; a model to assess efficacy of disinfectants against hepadnavirus infectivity. Epidemiol Infect 1991;106:435–43.
[35] Willkommen H. Erfahrungen mit der Praxis von Infektionsassays und Modellviren für die Beurteilung der Sicherheit von Blutprodukten für den Patienten. In: Rabenau HF, Thraenhart O, Doerr HW, editors. Nosokomiale Virusinfektion—Erkennung und Bekämpfung. Lengerich: Pabst Science Publishers, 2001; 166–73.
[36] Borovec S, Proumis C, Adcock W, Fang R, Uren E. Inactivation kinetics of model and relevant blood-borne viruses by treatment with sodium hydroxide and heat. Biologicals 1998;26(3):237–44.