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ORIGINAL PAPER

Bacterial contamination of amniotic membrane in a tissue bank from Iran

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Abstract Human Amniotic Membrane (AM) transplantation can promote tissue healing and reduce inflammation, tissue scarring and neovascularization. Homa Peyvand Tamin (HPT) tissue bank has focused on manufacturing human cell and tissue based products including AM. The purpose of this study is to evaluate and identify bacterial contamination of AMs that is produced by HPT for several ophthalmic applications. From July 2006 to April 2011, 122 placentas from cesarean sections were retrieved by HPT after obtaining informed consent from the donors. Besides testing donor's blood sample for viral markers, microbiological evaluation was performed pre and post processing. During tissue processing, decontamination was performed by an antibiotic cocktail including; Gentamicin, Ceftriaxone and Cloxacillin. Of 271 cesarean section AM donors who were screened as potential donors, 122 were accepted for processing and assessed for microbiological contamination. Donors' age were between 21 and 41 years (Mean = 27.61 ± 0.24). More than 92 % of mothers were in their first or second gravidity with full term pregnancies. The most prevalent organisms were *Staphylococci* species (72.53 %). After processing, contamination rates markedly decreased by 84.62 % (p value = 0.013). According to our results, most of bacterial contaminations were related to donation process and the contamination pattern suggests procurement team as a source. Therefore we recommend that regular training programs should be implemented by tissue banks for procurement staff. These programs should focus on improved donor screening and proper aseptic technique for tissue retrieval. We also suggest that tissue banks should periodically check the rate and types of tissue contaminations. These data help them to find system faults and to update processing methods.

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Introduction

Human amniotic membrane (AM) is the innermost layer of the fetal membranes that consists of an epithelial layer on a basement membrane with an avascular stromal matrix (Tseng 2001). AM transplantation can promote tissue healing, reduce inflammation, minimize tissue scarring and eliminate neovascularization. It has some unique properties including the facilitation of epithelial cell migration, reinforcement of basal cell adhesion, induction of epithelial differentiation, and decreasing inflammation and bacterial infection (Muraine et al. 2001; Chen et al. 2000; Sangwan et al. 2007). The revival of AM transplantation in ophthalmology was happened in the past decades. In ophthalmology, it can be used as a temporary graft in order to decrease inflammation and promote re-epithelialization or as a permanent graft to replace damaged corneal and conjunctival tissue. Despite several reports on the beneficial role of AM in treating a variety of ocular disorders, its use was abandoned for five decades up to 1995 when Kim and Tseng reintroduced it to ophthalmology (Kim and Tseng 1995). Recently AM has been used for epithelial growth in treatment of persistent epithelial defects (Chen et al. 2000) and also for ex vivo cultivation of limbal and corneal epithelial cells (Sangwan et al. 2007). Furthermore, it has been successfully used in corneal disorders such as neurotrophic ulcers, persistent epithelial defects, shield ulcers, microbial keratitis, band keratopathy, bullous keratopathy, chemical injury and ocular surface reconstruction (Baradaran-Rafii et al. 2007). AM will be an ideal graft for the ocular surface reconstruction if some safety concerns can be solved (Prabhasawat et al. 2000). The risk of bacterial infection and disease transmission through tissue transplantation is one of the most important concerns of tissue transplantation (Wang et al. 2007). Many standards and guidelines have been developed to increase the safety and efficacy of tissue grafts. The main focus of them is implementation of quality management system in tissue establishments. Among different sterilization and decontamination methods, antibiotic treatment is the method of choice for cryopreserved AM. However, it is only effective against bacteria and fungi, and its effectiveness is depending on the type and concentration of antibiotic (Adds et al. 2001). Therefore, providing an acceptable level of safety is a fundamental issue in tissue manufacturing. Serological examination, microbiological evaluation, environmental monitoring and biosafety controls are crucially important in quality assurance programs since microbiological contamination of donor material before or during the manufacturing can potentially present a serious hazard to recipients (Cobo et al. 2005). The safe release of tissues for clinical use is an important priority and a great responsibility for the professionals in this field. Three main origin of tissue contamination are: donor, environment, and operator (Vicentino et al. 2009). Homa Peyvand Tamin (HPT) that was established in 2007, has focused on human cell and tissue based products manufacturing and its first product was cryopreserved human AM. HPT adheres to strict policies and standards according to European Association of Tissue Banks (EATB) and good manufacturing practice (GMP) in all activities and procedures (Aghayan et al. 2010).The purpose of this study was to evaluate microbial contamination of cryopreserved AM which were processed by HPT for various clinical applications in ophthalmology. To our knowledge, this is the first series of reporting the contamination rate of donated AM with a large number of subjects in a tissue bank.

Materials and methods

From July 2006 to April 2011, 271 AM donors from cesarean sections were referred to HPT that 149 donors were excluded according to donor selection criteria. Strict screening was performed for each potential donor including physical examination, medical and social history. Tissues from eligible donors were retrieved after obtaining informed consent. A satellite blood sample was obtained from each donor with venipuncture, and the sera were isolated by centrifugation and sent to a reference laboratory to be assessed for hepatitis B surface antigen (HBs-Ag), hepatitis B core antibody (HBc-Ab), antibodies against hepatitis C virus (HCV-Ab), human immunodeficiency virus ($HIV_{1,2}$-Ab), human T-lymphotrophic virus ($HTLV_{1,2}$-Ab) and RPR by ELISA technique (all from BioKit-Spain). Additionally, HCV and HIV

were checked by polymerase chain reaction (PCR) method (Roche, Germany). Human placentas were kept in sterile plastic bags or bottles on ice during transfer to the tissue bank, and were processed under class 100 biosafety cabinet which was located in a clean room. Microbiological sampling was performed for aerobic and anaerobic bacteria and fungi from transportation solution and pieces of membrane before and after antibiotic treatment. Under biosafety cabinet, samples from each quadrant of AM were taken for culture with a swab stick, and then these samples were transported in thioglycolate media to a reference laboratory. Microbiological evaluations were done for tissue pieces and also transportation solution separately. Briefly, for aerobic and anaerobic bacteria samples were incubated in thioglycolate culture media, whereas for fungi incubation was performed in a sabouraud dextrose agar culture medium. Incubation temperatures ranged between 24 and 37 °C. Samples were monitored by the laboratory for 14 days, with a daily control for visual evidence of growth (turbidity). Positive cultures were Gram stained and transferred at selective media (blood-enriched agar-medium, Mac Cockney medium and Chapman medium) to identify the genus and species. For microbial decontamination we used antibiotic solution including Gentamicin, Ceftriaxone and Cloxacillin. The preservative medium was 1:1 volume of glycerol (MP Biomedicals, USA) and Dulbecco's Modified Eagle Medium (DMEM; Biosera, UK).

Results

122 out of 271 AM donors were assessed by microbiological tests. Donors' age were from 21 to 41 years

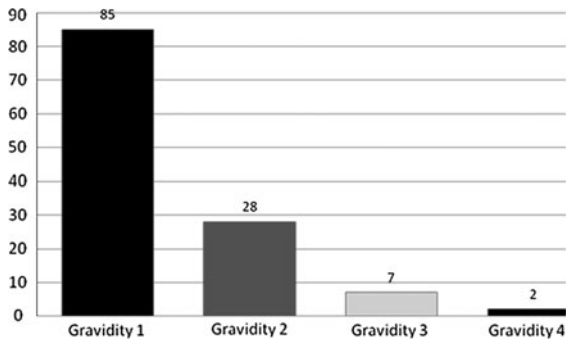

Fig. 1 Gravidity of amniotic membrane donors

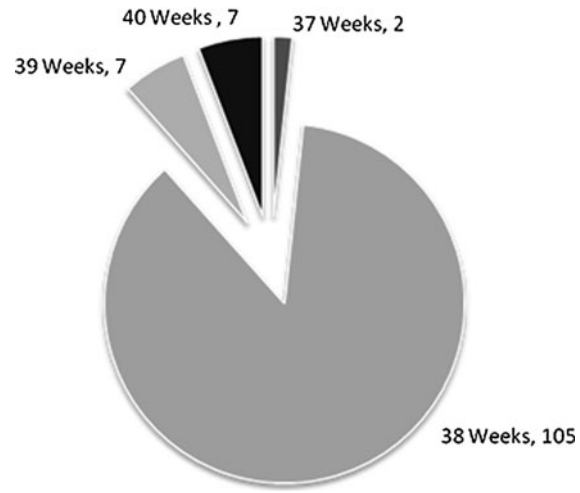

Fig. 2 Gestational age of amniotic membrane donors

(Mean = 27.606 ± 0.24). Number of mothers' gravidity is shown in Fig. 1 and Fig. 2 illustrates the gestational age of donors. The rate of microbial contamination in retrieved tissues was 61 %. As mentioned in method section, pre-processing samples were taken from transportation solution and tissue pieces. 51.35 % of positive samples were resulted from contaminated transportation solutions and 32.43 % from contaminated tissues, while in 16.22 % both tissue and solution were positive. The most prevalent organisms were *Staphylococci* species (72.53 %). After processing, contamination rates markedly decreased by 84.62 % (p value = 0.013). Different organisms that were identified by microbiological studies are demonstrated in Table 1.

Discussion

Before transplantation of tissue grafts, it must first be rendered free of microbial contamination. This requires aseptic tissue handling and an effective decontamination process. (Adds et al. 2001). Safety concern is an important issue in tissue banking and avoiding viral and microbial contamination during tissue procurement and processing is the major priority of tissue manufacturers. Tissue procurement under clean conditions can minimize the contamination risk and increase tissue and recipient safety. The recipient infection can be caused by transplantation of tissue which has been contaminated during tissue retrieval, processing, preservation and handling or at

Table 1 Different organism species identified with microbiological tests

Organism	Before antibiotic treating	After antibiotic treating	*p* Value
Staphylococcus warneri	25 (20.49 %)	0 (0 %)	0.019$^{\psi}$
Staphylococcus hominis	23 (18.85 %)	2 (1.63 %)	0.027$^{\psi}$
Propionibacterium acnes	11 (9.01 %)	4 (3.27 %)	0.049$^{\psi}$
Staphylococcus epidermidis	8 (6.55 %)	2 (1.63 %)	0.279†
Staphylococcus aureus	7 (5.73 %)	0 (0 %)	0.233†
Bacillus (Gram Positive)	5 (4.09 %)	1 (0.81 %)	0.694†
Coryneform bacteria	5 (4.09 %)	0 (0 %)	ND*
Enterococcus species	2 (1.63 %)	0 (0 %)	ND*
Staphylococcus hemolyticus	2 (1.63 %)	0 (0 %)	ND*
Staphylococcus schleiferi	1 (0.81 %)	0 (0 %)	ND*
Staphylococcus simulans	0 (0 %)	1 (0.81 %)	ND*
Candida species	1 (0.81 %)	0 (0 %)	ND*
Aspergillus	1 (0.81 %)	0 (0 %)	ND*
Anaerobics	0 (0 %)	4 (28.57 %)	0.585†
Total	91 (100 %)	14 (100 %)	0.013$^{\psi}$

* Not detected

$^{\psi}$ Significant difference

† No significant difference

transplantation time. According to tissue banking standards (American Association of Tissue Banks 2002), bacterial control of tissue grafts before and during the processing, can be effective in graft sterility at the time of implantation. Previous clinical studies on infection after AM transplantation demonstrated gram-positive organisms (especially *Staphylococcus* species) as the most frequent agents (Khokhar et al. 2001; Marangon et al. 2004). In this study we checked microbial contamination pre and post tissue processing. The rate of contamination in our study (61 %) was lower than the previous reported rates (100 %) by Souza et al. (2004) and Adds et al. (2001). It can be concluded that strict donor screening, procurement team training and effective relation with the hospitals (as tissue suppliers) have an important role in decreasing contamination rate of tissue products. Similar to the mentioned previous studies, we identified Staphylococci species as the most frequently isolated agents. More than 90 % of pre-processing bacteria were *Staphylococci* species, *Propionibacterium* acnes and *Coryneform* bacteria that are normal skin flora. As the intra-operative contamination is common, it can increase the contamination risk via donor or personnel normal flora. (Davis et al. 1999; Hughes and Anderson 1999). The most predominant bacteria from pre-processing samples were *Staphylococcus* warneri, a gram-positive bacterium that normally found on the skin of humans and animals, in nasal cavity and in the mouth. These types of *Staphylococci* are contaminants of medical devices like catheters, prosthesis of various types, and artificial and native heart valves and can induce nosocomial infections of immune-compromised patients and neonates (Incani et al. 2010). The major sources of these organisms are patients' skin or nasal flora, airborne particles from operating theatre personnel (Haeri and Wiley 1980; Howorth 1985), hematologic seeding after device implantation, or some break in aseptic technique (Levy et al. 1990; Edmiston 1993; Centers for Disease Control and Prevention 1999; Seabrook and Edmiston 2001). Davis et al. (1999) showed that materials are frequently contaminated during elective orthopedic surgery by the theatre gowns and the gloves of the operating team. Howorth (1985) described that, *Staphylococcus* aureus and the coagulase-negative *Staphylococci* are the predominant organisms associated with contamination of implantable biomedical devices. We demonstrated that in 51.35 % of total positive results the solutions were contaminated whereas the tissue cultures were negative. Therefore, besides tissue sampling, evaluation of transportation solution is crucial to determine pre-processing sources of contamination. All of our donors were selected from elective cesarean sections and full term pregnancies that most of them (86 %) were in 38th week and others were in 39th to 40th week of gestational age. Also about 70 % of mothers were primiparous. Adds et al. (2001) reported that 12 different species were recovered from 10 cesarean

samples but we identified 8 species in our samples. Table 1 demonstrates that, 91 microorganisms were isolated pre-processing and 14 post-processing. This significant decrease in the rate of post processing contaminations may be related to the effective tissue preparation method including; antibiotic treatment, tissue trimming, and multiple washing steps. On the other hand, we selected the type and concentration of antibiotics based on bacterial prevalence and their antibiotic resistance. We found that five negative pre-processing samples were positive after tissue processing. It can be related to personnel fault during processing, improper sampling, contamination via instruments or some break in aseptic techniques. The authors recommend that regular training programs should be implemented by tissue banks for procurement staff. These programs should focus on improved donor screening and proper aseptic technique for tissue retrieval. We also suggest that tissue banks should periodically check the rate and types of tissue contaminations and analyze this information. The resulting data can help them to find system errors and to update processing methods. To improve recipient safety, authors suggest that any tissue which is contaminated with fungi or bacteria with high pathogenicity should be discarded even if post-processing culture is negative.

Conclusion

There is a serious risk of microbial contamination of AM before or during tissue processing. Like previous studies, in the current study the most frequent organisms were skin commensals that probably represent external contamination at the time of procurement. Therefore it is necessary to improve donor screening, tissue processing and decontamination methods. Also to achieve an acceptable level of tissue safety and quality, tissue banks should adhere to more strict standards in their procedures. According to the results of current study, most of bacterial contaminations were found before processing. Therefore, in addition to other parts of quality management programs, training of procurement staff can help tissue bankers to reduce tissue contamination rates.

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