JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2013. Vol. **7**(1), p. 329-334

Prolonging Living Tissue and Organ in Dry State through Anhydrobiosis

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(Received: 10 January 2013; accepted: 24 February 2013)

To survive in desiccation, a phenomenon known as "anhydrobiosis" is widely used by some simple organisms, such as water bear and larvae of sleeping chironomid. From previous experience, we know that the ability to survive in anhydrobiosis or in dry state is correlated with the accumulation of trehalose. Recently, several new reports have demonstrated that endogenous and exogenous trehalose has also been used to increase desiccation tolerance of mammalian cells. Herein, we postulate that trehalose can be injected into donated tissue and organ in the dry state as a method for long-term storage and transportation of liveng tissue and organ. Trehalose can be introduced into cells of tissue and organ through the trehalose-containing perfusion medium. These trehalose-loaded tissue and organ can be dried and stored at room temperature under vacuum.

Key words: Trehalose, Drying preservation, Anhydrobiosis.

Larvae of sleeping chronomid, Ploypedilum vanderplanki, can survive in complete dehydration in a "cryptobiosis" or "anhydrobiosis"state (life without water). Larvae accumulate 18% of their dry body mass as trehalose, which is thought to play a key role in maintaining their vitality(Kikawada *et al*., 2007; Watanabe *et al.,* 2002). Water bear, a tardigrade living in the water film around soil grains, can be revived after extreme desiccation for decades. Trehalose was also found at high concentration in this microscopic anima(Crowe and Crowe, 2000; Hengherr *et al.,* 2008). Beside Larvae and water bear, many organisms such as yeast, bacteria, cyanobacteria rotifers, nematodes and some plant seeds demonstrate desiccation tolerance, which is related to the accumulate of trehalose(Carvalho *et al.,* 2011; Guo *et al.,* 2000; Hengherr *et al.,* 2008; McIntyre *et al.,* 2007).

Trehalose, a non-reducing disaccharide of glucose(Becker *et al.,* 1996; Behm, 1997; Iturriaga *et al.,* 2009), is a well known protector of biostructures like liposomes(Chen and Lu, 2006; Stoll *et al.,* 2012; Wieber *et al.,* 2012) and proteins(Jain and Roy, 2010) during freeze-drying. The mechanism of trehalose-mediated desiccation tolerance is probably due to the exogenously added trehalose reversibly replacing water molecules and compensating for the loss of hydrogen bonding between intracellular molecules and water(water

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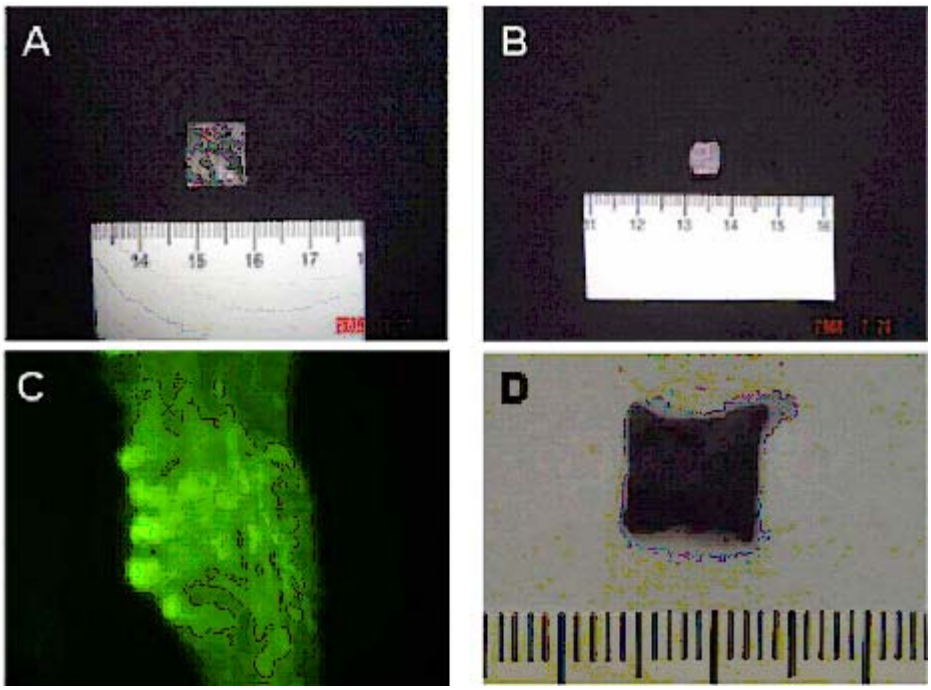
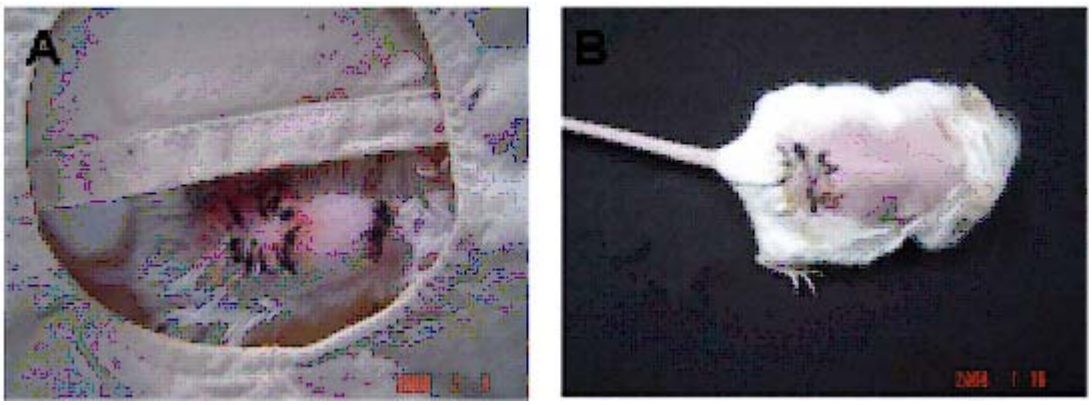
replacement hypothesis)(Golovina *et al.,* ; Horta *et al.,* 2010). It may also help the formation of a highly viscous liquid, termed stable glass, during drying(Puhlev *et al.,* 2001; Tablin *et al.,* 2001; Wolkers *et al.,* 2002).

Although mammalian cells could not produce trehalose(Teramoto *et al.,* 2008), endogenous and exogenous trehalose was found to significantly improve desiccation tolerance of mammalian cells introduced with this disaccharide(Campbell and Brockbank, 2012; Chen *et al.,* 2001; Gordon *et al.,* 2001; Guo *et al.,* 2000; Wolkers *et al.,* 2001). Specifically, mouse sperm were dried for 5 min in EGTA medium with 0.5M trehalose and stored for 1 month at 4°C,after rehydration, they were injected into oocytes and delivered three live fetuses(McGinnis *et al.,* 2005). Meanwhile, the viability of tissue and organ varied



A: Dried mouse skin after introducing trehalose, B: Mouse skin after rehydration, C: Mouse skin after CFDA dyeing, D: Mouse skin after MTT dyeing

Fig. 1. Mouse skin



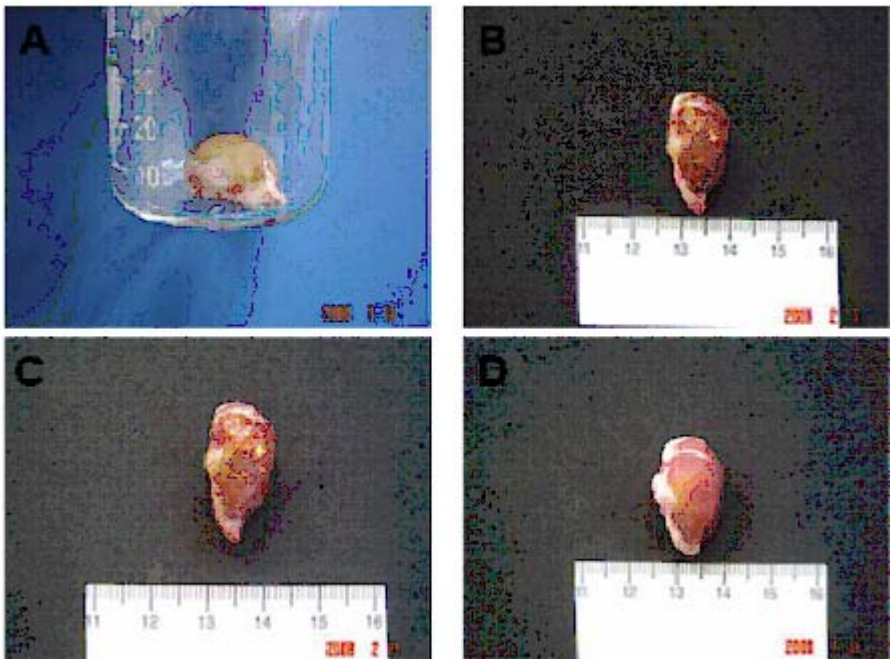
A: Recovered mouse skin were autoplastic transplantation, B: Recovered mouse skin were allotransplantation for 5 days

Fig. 2. Mouse skin transplantation

from several hours to several days after donation(Stowe *et al.,* 2007). The donated tissue and organ were usually preserved and transported in cold condition after perfusion. However, because of frequent mismatch of HLA to registered patients and the technical difficulty in long time preservation of live tissue and organ, many donated organs fail to be transplanted(Maathuis *et al.,* 2007). Presently, researchers have yet to apply trehalose in preserving live tissue and organ in dry state.

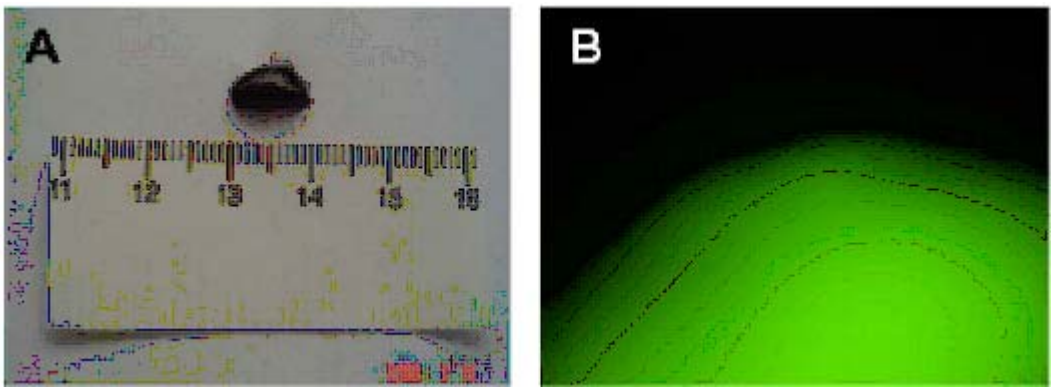
Presentation of hypothesis

We postulate that trehalose may have the same effect(improve desiccation tolerance) on mammal tissue and organ preservation as it does on simple organisms. Donated tissue and organ



A: Rabbit kidney after perfusion with DMEM medium containing 500mM trehalose, B: Rabbit kidney after being freeze-dried, C: Rabbit kidney after prehydration, D: Rabbit kidney after rehydration, E: Rabbit kidney after CFDA dyeding, F: Rabbit kidney after MTT dyeing

Fig. 3. Rabbit kidney



A: Rabbit kidney after CFDA dyeding, B: Rabbit kidney after MTT dyeing.

Fig. 4. Rabbit kidney dyeing

which have been introduced with trehalose could be stored and transported at room temperature under vacuum for a long time without the loss of their initial functions after drying.

Theoretic fundamentals of the hypothesis

Endogenous trehalose preserve mammalian cells during desiccation Guo et al used a recombinant adenovirus vector, which expressed the otsA(encode trehalose 6-phosphate synthase) (Reina-Bueno *et al.,* 2012) and otsB(encod trehalose 6-phosphate phosphatase)(Li *et al.,* 2012; Wingler *et al.,* 2012), to infect human primary fibroblast and succeeded in maintaining live cells in the dry state for up to five days. This result revealed that trehalose could be used in mammalian cell preservation(Guo *et al.,* 2000).

Exogenous trehalose preserve mammalian cells during desiccation

Platelets have a average lifespan of 5 days and must be stored above 20°Cto retain their normal functions(Tablin *et al.,* 2001). In Tablin et al's experiment, platelets with a concentration of 1-2×10^9/ml, were incubated at 37°Cfor 4h in a buffer containing 35mM trehalose prior to freeze-drying and were stored at room temperature under vacuum, which resulted in a survival rate of over 90% after 7 months. These recovered platelets have similar function as fresh platelets, such as responding to thrombin, collagen, and ristocetin. Platelets from the control, without introducing trehalose, formed an insoluble clump and lost clinical value after rehydration(Tablin *et al.,* 2001; Wolkers *et al.,* 2002). Recently, dried human mesenchymal stem cells(hMSCs) were successfully shipped from San Diego to Baltimore overnight in a vacuum sealer. These cells were incubated in medium containing 50mM trehalose and 3% glycerol for 24h. hMSCs were then air-dried, stored in a vacuum sealer and shipped from San Diego to Baltimore at ambient temperature range from -1 to 18°C. The transported dry cells were rehydrated by normal medium and incubated at 37°C,5%CO_2. Over 90% of the cells retained their viability and phenotype(Gordon *et al.,* 2001). Finally, Lynda et al successfully produced live-born pups from mouse sperm desiccated for 5 min in a 0.5M trehalose solution and stored for 1 month at 4°C(McGinnis *et al.,* 2005).

Based on the above evidence, we postulate that the application of trehalose in organ preservation could be a novel method for long-term storage of tissue and organ. We could apply trehalose in the storage of donated tissue and organ. Trehalose could be introduced into tissue and organ with trehalose-containing perfusion medium. The tissue and organ loaded with trehalose could then be dried and subsequently stored and transported at room temperature under vacuum without the loss of natural functions. Furthermore, in engineered tissue regeneration protocol, we could introduce trehalose into cells by incubating cells in the presence of trehalose in medium. The engineered tissue composed of trehalose-loaded cells could then be stored at room temperature under vacuum for a long time after drying.

Additional remark

We have initially examined our hypothesis as follows:

Experiment 1

Mouse skin, at a size of 1×1cm, were previously incubated in DMEM medium containing 500mM trehalose at 37°C for 7h. Then they were freeze-dried (Figure 1A) and stored at room temperature under vacuum for 7 days. Next, dried mouse skin were rehydration (Figure 1B) and resuspended in CFDA solution(Figure 1C) and MTT solution(Figure 1D), indicating that recovered mouse skin remained viable. We then made autoplastic transplantation(Figure 2A) and allotransplantation(Figure 2B).

Experiment 2

Rabbit kidney, perfused with DMEM medium containing 500mM trehalose(Figure 3A), was freeze-dried(Figure 3B) and stored under vacuum for 5 days, then prehydration(Figure 3C) and rehydration (Figure 3D) were conducted. Recovered rabbit kidney was placed in CFDA solution(Figure 4A) and MTT solution(Figure 4B), which demonstrated that dried kidney can remain viable for at least 5 days.

Conflict of interests

I, Fulin Chen of Northwest University, declare on behalf of all co-authors of "Prolonging living tissue and organ in dry state through anhydrobiosis" that no conflicts of interest exist for this submission.

ACKNOWLEDGEMENTS

This work was financed by National Natural Science Funds of China (Grant No.30970745).

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