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Development of New Organ Preservation Solutions in Kyoto University

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Although lung transplantation (LTx) has been established as a therapeutic approach for end-stage respiratory failure, several problems remain to be solved. In addition to the serious problem of donor shortage, primary graft failure, which is mostly caused by ischemia-reperfusion injury, a serious problem, and represents one of the most frequent causes of early mortality. The development of a highly reliable organ preservation solution that reduces ischemia-reperfusion injury will improve the functioning of transplanted organs and alleviate the donor shortage. We first evaluated the importance of saccharides and electrolytes in the lung preservation solution. We proved the superiority of trehalose, a non-reducing disaccharide, and the efficiency of the extracellular-type (low potassium) ion composition, and we also developed an extracellular-type trehalose containing Kyoto (ET-Kyoto) solution. Furthermore, several agents for vascular endothelial protection were evaluated, and finally, a more effective solution named "new ET-Kyoto solution" was developed, by adding N-acetylcysteine, dibutyryl adenosine 3', 5'-cyclic monophosphate, and nitroglycerin to the "conventional" ET-Kyoto solution. The new ET-Kyoto solution enabled canine LTx to last up to 30 hours. ET-Kyoto solution has so far been used and produced good results in five clinical LTx throughout Japan and South Korea. Although it was initially developed for lung preservation, its effectiveness in the preservation of various organs/tissues, such as the trachea, kidney, skin/muscle flap, amputated digits, liver, and pancreas, has also been experimentally and clinically shown. In this paper, clinical and experimental findings with ET-Kyoto solution have been accumulated to further analyze its effect, safety, and chemical stability. We hope to provide ET-Kyoto solution as the standard organ/tissue preserving solution throughout the world.

Key Words: Lung transplantation, preservation solution, lung preservation trehalose

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INTRODUCTION

Current trends in lung transplantation

Since the first successful operation by the Toronto Group in 1983,[1] lung transplantation (LTx) has been established as a therapeutic approach of choice for end-stage respiratory failure. To date, more than 17,000 LTx have been performed all over the world. The 5-year survival rate after LTx is around 50% according to the international survey,[2] being almost satisfactory as a therapeutic option for end-stage respiratory failure with no other options. However, several problems need to be solved. In Japan, the organ transplantation law was enforced in 1997, and the first cadaveric organ transplantations for the heart, liver, and kidney were respectively performed in 1999. However, donors suitable for LTx have waited a long time until the first cadaveric LTx in 2000. Meanwhile, the first living-donor lobar LTx was performed in 1998. Throughout September 2004, 18 cadaveric LTx and 41 living-donor lobar LTx have been performed in Japan. During this period, organs were supplied for transplantation from 31 brain-dead donors, but the lungs were available in only 16 donors. In cadaveric LTx as well as living-donor lobar LTx, the outcome in Japan seems to be better than that in European and North American countries.[2] However, the number of LTx remains low, and there are more living-donor lobar LTx than cadaveric LTx in Japan due to the severe shortage of cadaveric donors.

Problems of lung transplantation

There are many complications after LTx, including primary graft failure, acute or chronic rejection, infection due to immunosuppression, surgical complications, especially healing impairment at the site of bronchial anastomosis, and secondary malignant tumors.[3]

Among them, primary graft failure occurs in the early postoperative days and represents one of the most frequent causes of early mortality during the first 30 days.2 In most cases, primary graft failure is caused by ischemia-reperfusion (I-R) injury, that is, injury due to the interruption (ischemia) and reopening (reperfusion) of the blood flow to the organ.[4] Therefore, the development of a highly reliable organ preservation solution to reduce I-R injury will improve the function of the transplanted organ and the outcome of LTx itself.

The shortage of brain-death donors is also a grave problem. Nowadays, more than 1,500 LTx are annually performed throughout the world, but its number has recently reached a plateau.[2] Due to the increasing number of patients on waiting lists and the shortage of donor lungs, the waiting time for all patients has been prolonged with heightened "on the list" mortality. In Japan, from 1998 through September 2004, 187 patients with end-stage pulmonary diseases have been registered on the list of the Japan Organ Transplant Network. Fifty-nine (31.6%) among them died without LTx, and the mean waiting time for the 18 cases of cadaveric LTx in Japan was 19 months. In European and North American countries, it was more than 18 months.[5,6] However, the waiting time in Japan may lengthen the near future because LTx was introduced to Japan only a few years ago. Moreover, considering the very high ratio of living-donor lobar LTx to cadaveric LTx, the overall situation concerning LTx is more severe in Japan than in European and North American countries.

This shortage of donor lungs partly results from the lack of social recognition of transplantation and brain-death donation in Japan. Since the ischemic time should be within 10 hours for LTx,[7,8] donor lungs from brain-death donors from a long distance or in bad condition (i.e., marginal donor lungs) cannot be used for LTx, which also worsens the shortage of donors.

Primary graft failure due to I-R injury and donor shortage are common not only in LTx, but also in the transplantation of other organs, such as the heart, liver, kidney, pancreas, and the small intestine.

Therefore, the development of a highly effective and reliable organ preservation solution will contribute to improve the function of transplanted organs and to alleviate the shortage of donor organs.

Moreover, I-R injury may induce rejection, which is the principal cause of mortality after transplantation.[9] Therefore, a highly effective organ preservation solution might also help to reduce the occurrence of organ rejection.

Development of ET-Kyoto solution

Recent development in organ preservation solution

Various efforts such as the development of new immunosuppressive agents have contributed to improve the outcome of organ transplantation. However, since the Euro-Collins (EC) solution and University of Wisconsin (UW) solution came into worldwide use, few studies on the development of organ preservation solutions have been performed in Japan or throughout the world.

Since its first clinical application for renal transplantation,[10] EC solution has been widely used not only in the transplantation of abdominal organs, such as the kidney and the liver, but has also been widely used for lung transplantation. Moreover, UW solution, which was first developed as a preservation solution for the liver, kidney, and pancreas, has also prevailed in lung transplantation. The current trend for lung preservation is to flush the pulmonary vasculature with cold modified EC solution supplemented by prostaglandins or with UW solution. The maximum ischemic period at most transplantation centers with such solutions is ten hours.[7,8] However, many centers are not satisfied with the quality of grafts preserved with EC solution.

To produce a more reliable preseravtion solution, we first evaluated the importance of saccharides and electrolytes in lung preservation and developed our original ET-Kyoto solution (Table 1). Furthermore, some agents were evaluated for

Table 1. Organ Preservation Solutions

Component		Effect	EC	UW	LPDG	EC-3.5T	EC-7T	ET-K	IT-K	New ET-K
Na^+	(mM)	cation	10	30	165	10	10	100	20	107
K^+	(mM)	cation	115	125	4	115	115	44	130	42
Cl^-	(mM)	anion	15		101	15	15			
Gluconate	(mM)	anion						100	106	97
Mg^{++}	(mM)	cation		5	2					
Sulfate	(mM)	buffer		5	2					
Bicarbonate	(mM)	buffer	10			10	10			
Phosphate	(mM)	buffer	58	25	34	58	58	25	25	24
Lactobionate	(mM)	buffer		100						
Glucose	(mM)	sugar	194		56					
Trehalose	(mM)	sugar				100	200	120	120	120
Raffinose	(mM)	sugar		30						
HES	(g/L)	colloid		50				30	30	29
Dextran 40	(g/L)	colloid			20					
N-acetylcysteine	(mM)	ROS scavenger								10
Dibutyryl cAMP	(mM)	2nd messenger								2
Nitroglycerin	(mM)	NO donor								0.44
Adenosine	(mM)	energy source		5						
Allopurinol	(mM)	ROS scavenger		1						
Glutathione	(mM)	ROS scavenger		3						
Osmolarity	(mOsm)	355	320	335	271	373	366	370	598	

EC, euro-collins; UW, University of Wisconsin; LPDG, low potassium dextran glucose; EC-3.5T, EC with trehalose (3.5%); EC-7T, EC with trehalose (7.0%); ET-K, ET-Kyoto; IT-K, IT-Kyoto; new ET-K, new ET-Kyoto; HES, hydroxyethyl starch; OS, reactive oxygen species.

the protection of vasculature, and eventually, we developed a more effective solution named "new ET-Kyoto solution", which enabled 30 hours of lung preservation.

Role of saccharides in lung preservation and trehalose

In hypothermic organ preservation, the function of the Na^+ pump (Na-K ATPase) decreases. Na^+ and Cl^- flow with water from the extracellular space into the intracellular space according to the gradient of ion concentration, resulting in cellular edema. In the field of organ preservation, saccharides are usually considered to prevent this cellular edema by acting as an impermeant and energy source during ischemia. However, the best saccharide to use in organ preservation solutions has not yet been identified.

Trehalose is a non-reducing disaccharide that is composed of two D-glucose moieties connected by a 1, 1-linkage. It exists widely in nature and can be dissolved into glucose by hydrolysis. Trehalose is found in abundance in many prokaryotes, fungi, yeasts, some desert plants, and body fluid of insects.[11] It has been reported to protect cells under various non-physiological conditions such as desiccation, freezing and high temperatures by stabilizing the cell membrane and creating a stable environment around the cells.[12,13]

We hypothesized that trehalose might protect the cell membrane against hypothermia and ischemia during organ preservation, and investigated its effect in lung preservation solutions. We prepared a modified-EC solution (EC-3.5T solution and EC-7T solution) in which glucose was (3.5% and 7.0%, respectively) replaced by trehalose, and the effect of trehalose on organ preservation was studied using a 12-hour canine lung preservation and transplantation model.[14] Both EC-3.5T and EC-7T solutions had better preservation effects than the original EC solution con-

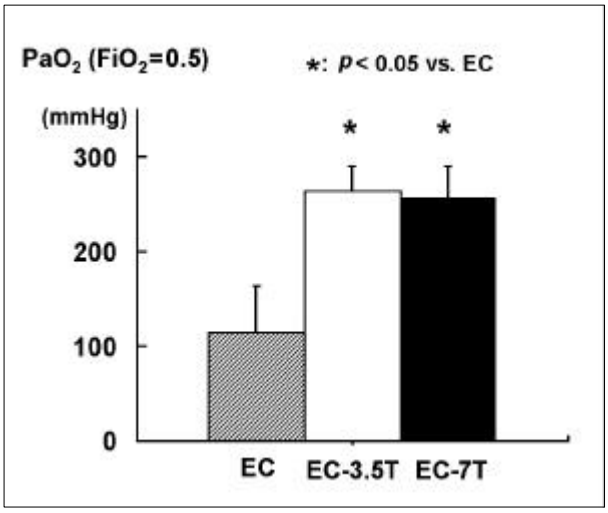

Fig. 1. PaO_2 (mmHg, FiO_2=0.5) 130 minutes after reperfusion in a 12-hour canine lung preservation and transplantation. PaO_2 in the EC-3.5T and the EC-7T groups were significantly higher than in the EC group ($p<0.05$). EC-3.5T, modified Euro-Collins (EC) solution in which 3.5% trehalose was used instead of glucose; EC-7T, modified EC solution in which 7% trehalose was used instead of glucose; EC, original EC solution.

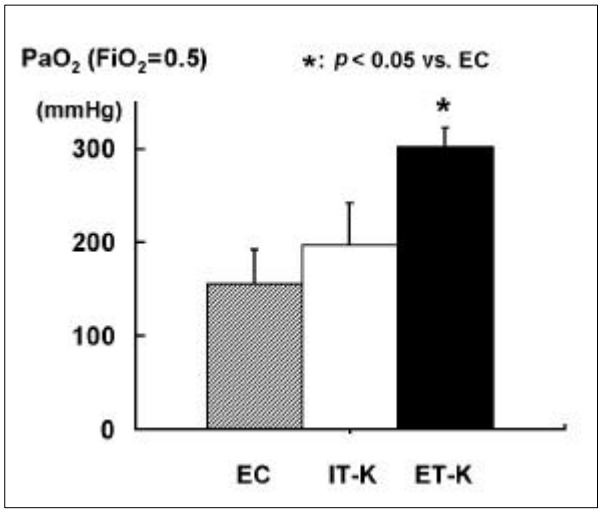

Fig. 2. PaO_2 (mmHg, FiO_2=0.5) 130 minutes after reperfusion in a 20-hour canine lung preservation and transplantation. PaO_2 in the ET-K group was significantly higher than in the EC group ($p<0.05$). ET-K, ET- Kyoto solution; IT-K, IT-Kyoto solution; EC, Euro-Collins solution.

taining glucose (Fig. 1). Furthermore, we confirmed that the appropriate concentration of trehalose is not as high as 10% but between 3.5% and 7% (unpublished data).

We also investigated the effects of various saccharides using an isolated rat lung perfusion model.[15] For saccharides in lung preservation, raffinose, a trisaccharide present in the UW solution, has been reported to have a cytoprotective effect.[16] The effects of a monosaccharide (glucose), disaccharides (trehalose, maltose, sucrose), and a trisaccharide (raffinose) in lung preservation were compared. In this study, trehalose was shown to be superior to other saccharides, and it was suggested that the protective effects of saccharides may depend and vary on their cytoprotective effect rather than on their activity as an impermeant or energy source.[15]

Extracellular type ET-Kyoto solution and 20-hour lung preservation

In clinical liver and kidney preservation, intracellular type (high potassium) preservation solutions have been used frequently since the development of the Collins' solution for kidney preservation.[10] However, we previously reported that a higher potassium level in intracellular type preservation solutions might result in the constriction of pulmonary arteries.[17] Therefore, intracellular-type IT-Kyoto solution (Na^+: 20 mmol/L, K^+: 130 mmol/L) and extracellular-type ET-Kyoto solution (Na^+: 100 mmol/L, K^+: 44 mmol/L) were compared to show which type is the better lung preservation solution.

IT-Kyoto and ET-Kyoto solutions also contain gluconate and hydroxyethyl starch (HES). Gluconate was used as an anion in place of chloride. The chloride ion passes freely through the cell membrane to draw water into the cell, but the cell membrane is much less permeable to gluconate due to its greater molecular weight.[18] HES was used to create sufficient osmotic pressure,[19] and phosphate was used as a buffer.

The efficacies of ET-Kyoto, IT-Kyoto, and EC solutions were examined in a 20-hour canine lung preservation and transplantation.[20,21] In these studies, ET-Kyoto solution provided a significantly better preservation quality than EC solution, and enabled a 20-hour long canine lung preservation (Fig. 2).

With transmission electron microscopy (TEM), less injury was suffered by the vascular endothelial cells in lungs that were preserved in ET-Kyoto solution compared to those stored in IT-

Kyoto solution.[22] We also evaluated the viability of murine endothelial cells stored in several preservation solutions, showing that ET-Kyoto solution is superior to UW and IT-Kyoto solutions.[23]

Optimal potassium concentration in preservation solution

The optimal potassium concentration in a preservation solution was further investigated. In a 48-hour canine lung preservation and transplantation model, the results showed that a potassium concentration at 44 mEq/L does not cause the deterioration of endothelial cells in comparison with that at 20 mEq/L.[24] In another experiment by our group, EC-based solution with the potassium concentration at 40 mEq/L was better than solutions with potassium at 115 mEq/L and 10 mEq/L in lung preservation. Thus, a medium (around 40 mEq/L) potassium concentration seems to be optimal in lung preservation solutions.[25]

Development of new ET-Kyoto solution

Supplement for vascular endothelial protection and new ET-Kyoto solution

Although, ET- Kyoto solution enabled a 20-hour canine lung preservation, it did not satisfactorily preserve the organ in a 30-hour canine lung preservation and transplantation model (data not shown). In view of the assessment with TEM, we considered that protection of the vascular endothelial cells was critical to preserve pulmonary function after transplantation.[22,26] Therefore, agents which protect the vascular endothelium were added, and the "new ET-Kyoto" solution was developed.

In organ preservation, the integrity of the vascular endothelium is a critical factor. In the vascular endothelial cells, I-R injury induces the depletion of adenosine 3′, 5′-cyclic monophosphate (cAMP), nitric oxide (NO), intracellular second messengers. It has been suggested that cAMP may protect the vascular endothelial barrier properties and suppress reactivity between vascular endothelial cells and neutrophils in the blood stream. Through the production of guanosine 3′, 5′-cyclic monophosphate (cGMP), NO also plays an important role in modulating the impermeability of the vascular endothelial cells, the interaction between vascular endothelial cells and neutrophils/platelets, and vascular resistance. In these contexts, the supplementation of cAMP and an NO donor to an organ preservation solution may protect vascular endothelial cells and allow for better organ function after transplantation.

Dibutyryl adenosine 3′, 5′-cyclic monophosphate (db-cAMP) is a membrane-permeable cAMP analogue that elevates intracellular cAMP levels. It acts as a vasodilator and protects the vascular endothelium.[27] Nitroglycerin (NTG), a donor of NO, increases intracellular NO and cGMP levels and dilates the pulmonary arteries.[28]

The protective effect of db-cAMP in lung preservation have been previously shown using the isolated rat lung perfusion models.[29,30] An ultrastructural examination with TEM showed that db-cAMP ameliorates damage to the vascular endothelial cells after cold storage of rat lungs.[31]

We also showed in the isolated rat lung perfusion model that NTG reduces oxidative stress and DNA damage in I-R of the lung and improves lung function after reperfusion.[30,32]

Lung preservation with new ET-Kyoto solution

The "new ET-Kyoto" solution was produced by adding N-acetylcysteine (NAC), db-cAMP, and NTG to the "conventional" ET-Kyoto solution to protect the vascular endothelium, and the new ET-Kyoto solution was evaluated for lung preservation. NAC, a scavenger for oxygen free radicals and a precursor of glutathione, has antioxidant activity, and we have already reported the protective effect of NAC in I-R injury of the lung.[33]

In a 30-hour process of canine lung preservation and transplantation, we showed that the new ET-Kyoto solution provides better preservation than UW solution[34] and low potassium dextran glucose (LPDG) solution[35] (Fig. 3 and 4). In the isolated rat lung perfusion model, pulmonary function after preservation with new ET-Kyoto solution was better than preservation with EC solution as well as LPDG solution, and was equal to that with UW solution.[25,36]

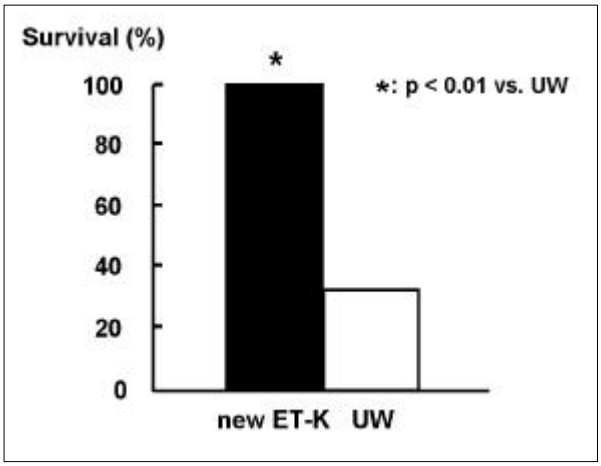

Fig. 3. Survival rate 6 hours after reperfusion (%) in a 30-hour canine lung preservation and transplantation. The survival rate in the new ET-K group was significantly higher than in the UW group ($p<0.01$). New-ET-K, new ET-Kyoto solution; UW, University of Wisconsin solution.

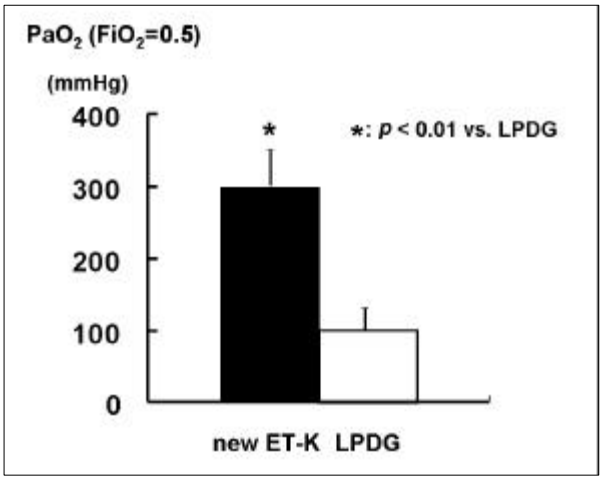

Fig. 4. PaO_2 (mmHg, FiO_2=0.5) 6 hours after reperfusion in a 30-hour canine lung preservation and transplantation. PaO_2 in the new ET-K group was significantly higher than in the LPDG group ($p<0.01$). New ET-K, new ET-Kyoto solution; LPDG, low potassium dextran glucose solution.

Application of ET-Kyoto solution in clinical lung transplantation

To date, ET-Kyoto solution has been used to produce good results in 5 cases of LTx (3 cases at Kyoto University, 1 case at another institution in Japan, and 1 case at Yonsei University in South Korea). In the 3 cases at Kyoto University, two cases were living-donor lobar LTx for bronchiectasis and lymphangioleiomyomatosis (LAM), respectively, and the other was a cadaveric LTx for pulmonary emphysema.

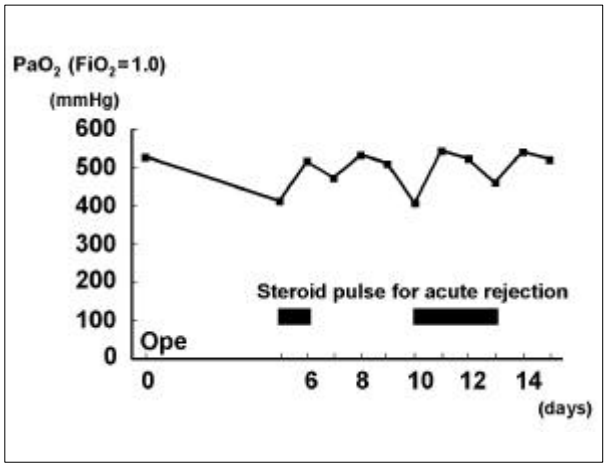

Fig. 5. Post-operative PaO_2 (mmHg, FiO_2=1.0) in a clinical case. Ope, operation.

In clinical application, we used ET-Kyoto solution with NTG and db-cAMP supplemented just before the pulmonary vasculature flush. Because the protective effect of NAC in cold lung preservation was not clearly shown (unpublished data), NAC was not used in clinical LTx.

We, herein, show brief data of the first clinical application of ET-Kyoto solution in LTx. The recipient was a 49-year-old female with diffuse bronchiectasis who underwent living-donor lobar LTx.[37] The donors were her husband and her son. The ischemic time was 248/141 minutes for the right/left lungs, respectively. Postoperatively, PaO_2 (FiO_2=1.0) was over 450 mmHg, and she maintained good pulmonary function (Fig. 5).

Application of ET-Kyoto solution for other organs

The effects of ET-Kyoto solution in organ preservation have been shown not only in the lung but also in other organs, experimentally and clinically.

Trachea

In a cryopreservation and autotransplantation experiment of the canine trachea, all animals survived for more than 2 months without complications after a 9-month cryopreservation period of the tracheas in the preservation solution containing trehalose.[38]

Kidney

In clinical renal transplantation, kidneys are usually preserved with EC or UW solutions. We compared ET-Kyoto solution in cold kidney preservation with EC and UW solutions.[39] ET-Kyoto solution achieved a more thorough and uniform initial vasculature flush than EC or UW solutions. Moreover, ET-Kyoto solution provided a better quality of preservation than EC solution and was similarly effective as UW solution. On the other hand, ET-Kyoto solution is chemically more stable than UW solution. Therefore, ET-Kyoto solution is the best preservation solution for the kidney.

So far, ET-Kyoto solution has been used in 12 cases of clinical renal transplantation with satisfactory results (unpublished data).

Skin/muscle flap and amputated digits

In rabbit skin and rat muscle flap storage models, we showed the effectiveness of trehalose in the preservation solution.[40-43] In a rabbit skin flap storage model, ET-Kyoto solution was superior than with EC solution in terms of preservation.[44]

Clinically, ET-Kyoto solution has been used in 9 cases of replantation of amputated digits, and all digits have survived well.

Other organs

Experimental evaluation of ET-Kyoto solution in the preservation of the liver and pancreas is underway in collaboration with other groups.

CONCLUSION

We have developed original organ/tissue preservation solutions, named ET-Kyoto solution and new ET-Kyoto solution. Although ET-Kyoto solution was initially developed for lung preservation, its effectiveness in the preservation of various organs has also been shown.

We now plan to accumulate clinical as well as experimental findings with ET-Kyoto solution to analyze its effect, safety, and chemical stability. We aim to develop the ET-Kyoto solution as the standard organ preserving solution throughout the world.

Moreover, ET-Kyoto solution is also being evaluated as a cell/tissue preserving solution in the field of regenerative medicine and bioscience.

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