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A NOVEL SYSTEM FOR SMALL BOWEL PRESERVATION — CAVITARY TWO-LAYER (UNIVERSITY OF WISCONSIN SOLUTION / PERFLUOROCHEMICAL) COLD STORAGE METHOD —

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INDEXING WORDS

small bowel transplantation ; small bowel preservation ; two-layer cold storage method

SYNOPSIS

Organ preservation is very important to accomplish successful organ transplantation. Unlike the kidney, liver, pancreas or heart, the preservation method of the small bowel has not been established yet. In this study, we tested 24-48hr-preservation of rat small bowel by a cavitary two-layer (University of Wisconsin solution (UW)/ perfluorochemical(PFC)) cold storage method (two-layer method (group 1)) in heterotopic rat segmental small bowel transplant model, and compared it with simple cold storage method in UW (group 3). In an attempt to clarify that the oxygenation of the graft by the two-layer method allows for 48hr-preservation, a cavitary two-layer method without oxygen bubbling (group 2) was also examined. The 7 day survival rates after 24hr-preservation were 100 %, 67 % and 86 % for group 1, 2 and 3, respectively. On the other hand, the 7 day survival rates for group 1, 2 and 3 after 48hr-preservation were 86%, 20% and 0%, respectively. Histological studies of grafts preserved by the two-

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Abbreviations : UW, University of Wisconsin solution; PFC, perfluorochemical; ATP, adenosine triphosphate.

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layer method (group 1) for 48hrs on the 7th day posttransplant showed almost normal architecture of the intestinal mucosa. This study demonstrated that the oxygenation of the small bowel during preservation by the cavitary two-layer method using UW allows for 48hr-preservation of rat small bowel. This method is simple and reliable and holds promise for clinical small bowel transplantation.

INTRODUCTION

Although several reports of long term survival after human intestinal transplantation have been published sporadically,[1,7,8,22,24] the result is not so satisfactory as the kidney, liver, pancreas or heart. Despite the recent major improvements in surgical techniques and the introduction of new immunosuppressive regimens, many difficult problems remain in this field. One of these problems associated with small bowel transplantation is the lack of a simple and reliable preservation method. Because of this reason, human intestinal grafts have to be transplanted as soon as possible after harvesting. However the prolongation of the preservation time is essential to permit unhurried graft procurement from cadaver donors and recipient preparation, until the actual implantation is carried out. Considering these facts, a simple, effective and reliable method of small bowel preserevation is essential in accomplishing successful transplantation. As supplying oxygen to the graft during preservation has an advantage in its own right, simple hypothermic storage in a hyperbaric oxygen chamber[14] and continuous perfusion method[25] were applied to small bowel preservation. However these methods are technically difficult and complicated, and perfusion method incurs the risk of greater vascular injury. With regard to supplying oxygen to the pancreas, the two-layer method is similar to the continuous perfusion method but simple, and more gentle for the pancreas[10,11] and we have succeeded in 72-96hr-preservation of canine pancreas.[6,9] Recently we have developed a new cavitary two-layer (UW / PFC) cold storage method, which is a modified two-layer method, made it possible to extend preservation time for 48hrs in rat heart.[12]

In this study, we applied this new cavitary two-layer method to small bowel preservation and assessed the ability of this method of extending the preservation time of rat small bowel in heterotopic transplant model.

MATERIALS AND METHODS

Perfluorodeclaine, one of the perfluorochemicals (PFCs) was a kind gift from Dr. K. Yokoyama (The Green Cross Corp., Osaka, Japan).

Animals

A syngeneic rat model for transplantation was used in order to eliminate the effects of either rejection or graft versus host disease. Male Lewis rats, weighing 250 - 350g, were purchased from Oriental Kobo Ltd., Osaka, Japan. They were housed in a specific pathogen - free animal facility, and were maintained on a 12hr light-dark cycle and fed with commercially available rat chow and water ad libitum.

Small bowel transplantation

Rats were fasted overnight with water ad libitum prior to surgery and were anesthetized with sodium pentobarbital (50mg／kg body weight) intraperitoneally. Small bowel transplantation was performed with a modified Monchik and Russell technique.[15] A 15cm segment of the jejunum was isolated on a pedicle that included the portal vein and superior mesenteric artery with aortic cuff. The aorta was ligated above and below the superior mesenteric artery and flushed with iced saline containing 10 U／ml heparin. The graft was quickly removed and the intestinal lumen was cleaned with saline containing 0.5 % gentamicin. Subsequently both the lumen and artery of the graft were flushed with cooled UW and then the graft was trasplanted immediately or after preservation. Under operative microscopy, revascularization was accomplished by end to side anastomosis of the donor's aortic cuff to the recipient's abdominal aorta and the portal vein to the inferior vena cava. Both ends of the graft were exteriorized and sutured to the abdominal wall.

Preservation method

The cavitary two-layer (UW／PFC) cold storage apparatus is shown in Fig. 1. After the graft was flushed with cooled UW, both ends of the bowel loop were closed by suture ligation. The intestinal lumen was filled with UW. Then the whole graft was completely immersed in the

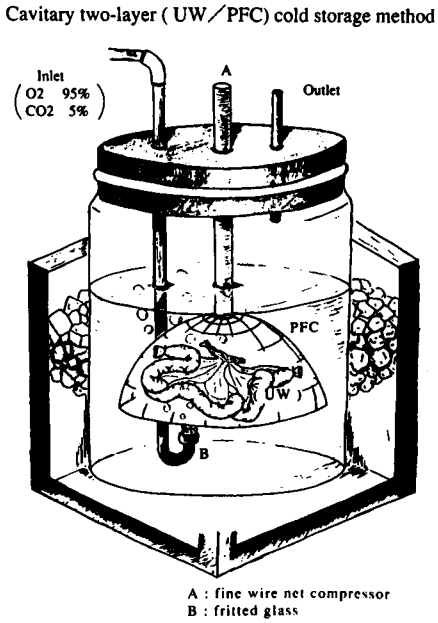

Fig. 1. The cavitary two-layer cold storage method. The small bowel graft contained of UW is immersed in PFC throughout the storage period, using a wire net compressor. Oxygenation was continued through the fritted glass to the PFC in a styrofoam box packed with ice.
(A : fine wire net compressor,
B : fritted glass)

PFC, using a wire net compressor. Without the compressor, the graft floated to the top of the PFC due to PFC's high specific gravity as described previously.[12] Oxygen : carbon dioxide (95% : 5%) was delivered continuously at a rate of 50-100 ml / min through a fritted glass to the PFC throughout the storage period. The temperature was kept at 4℃. Perfluorodeclain is chemically and biologically inert, colorless and dense, and has a high oxygen solubility of 40-50 vol%, approximately 10 times as much as water at 4℃. Therefore, it can be used as an oxygen carrying substance.

Assessment

All animals were inspected and their health was assessed daily until death. To evaluate the efficacy of preservation method, we examined 7day recipient survival and the histology at the 7th day after transplantation. All surviving animals were sacrificed on the 7th day after transplantation. The graft was removed and fixed in 10% formalin, paraffin- embedded, and stained with hematoxylin - eosin.

The mucosal damage was assessed with standard light microscopy. In another set of experiment, we also examined the histology of the graft immediately after preservation.

Exeperimental protocol

Four groups of rats were included in this study. There were 3 experimental groups in which all rats received segmental small bowel graft that was stored at 4℃ by the cavitary two- layer (UW / PFC) method with (group 1), or without oxygenation (group 2), or the simple cold storage method with UW (group 3). The groups were subdivided depending on the storage time : 1A,cavitary two-layer method with oxygenation for 24hrs.(n=5) ; 2A, cavitary two-layer method without oxygenation for 24hrs. (n=5) ; 3A, simple cold storage method with UW for 24hrs. (n=7) ; 1B,cavitary two-layer method with oxygenation for 48hrs. (n=7) ; 2B,cavitary two-layer method without oxygenation for 48hrs. (n=5) ; 3B, simple cold storage method with UW for 48hrs. (n=5). Another group was a control(group 4) (n=7) in which all rats received unpreserved segmental small bowell grafts.

RESULTS

7 days survival rate

After 24hr-preservation, the 7day survival rates of group 1A, 2A and 3A were 100% (5／5), 67 % (2／3) and 86% (6／7), respectively. In contrast, after 48hr preservation, survival rates of group 2B and 3B were 20% and 0% (1／5, 0／5) (Table 1). In all rats except one survived case in group 2B, intraluminal hemorrhage of the graft occurred immediately after reperfusion and most of these rats died within 24hrs after transplantation. Autopsies revealed graft necrosis. One of five rats in group 2B survived for 7days after transplantation. The graft was encapsulated and had dense adhesions, but the macroscopic vascular structure was maintained. On the other hand, after preservation by the cavitary two-layer method with oxygenation for 48hrs (group 1B), 7day survival rate was 86% (6 / 7). One of 7 rats in group 1B died of a massive hemorrhage from the graft mucosa 3days after transplantation. But, in other 6 rats in group 1B, the grafts became pinkish following temporary slight congestion and the animals were survived throughout the period of experiment.

Table I. 7 days survival after rat heterotopic segmental small bowel transplantation.

Group		Preservation method	Preservation time (hr)	7days survival (%)
1	A	UW /PFC + O_2	24	5/5 (100)
	B		48	6/7 (86)
2	A	UW /PFC	24	2/3 (67)
	B		48	1/5 (20)
3	A	UW	24	6/7 (86)
	B		48	0/5 (0)
control		—	0	7/7 (100)

Histology

Although all of the grafts were highly damaged from preservation and reperfusion injuries, histologic studies of group 1A from the biopsies taken 7 days after transplantation showed normal architecture of intestinal mucosa, just like control (group 4) (Fig. 2A and 2C). In groups 1B and 3A, almost normal architecture was observed except for a slight development of subepithelial Gruenhagen's space at tip of the villi (Fig. 2B and 2D). The histology of these grafts in groups 2B and 3B at autopsy showed hemorrhagic necrosis. We also examined the histology of the graft immediately after preservation. In groups 1A and 3A, the epithelial layer was lifting from the lamina propria down the sides of villi and a few tips were denuded (Fig. 3A and 3C). When the preservation period was longer, most of the villi were denuded with lamina propria and the remained villi were sparse in group 1B or 3B (Fig.3B and 3D).Though the degree of the graft damage was various indivisually,it was generally found that the longer the preservation period was, the severer the ischemic damage was. However no differencies were found between the two-layer storage group (group 1) and simple storage in UW group (group 3) in the same preservation time (1A and 3A, 1B and 3B).

DISCUSSION

A NOVEL SYSTEM FOR SMALL BOWEL PRESERVATION

Organ preservation is very important to accomplish successful organ transplantation. Unlike the kidney, liver, pancreas or heart, the preservation method of the small bowel has not been established yet. Raju et al. reported the 24hr canine small bowel preservation in cold Ringers-lactate with a success rate of 67%.[18] Regarding rat model, the small bowel can be preserved in good condition for up to 24hr using simple cold storage in UW solution[23] However, 24hr-preservation of small bowel in animal model is out of margin of clinical safety and longer preservation time is necessary. Based on the concept that graft oxygenation may contribute to prolonged small bowel preservation, Lillehei et al. reported simple hypothermic storage of canine small bowel[13] and succeeded in 48hr-preservation using a combination of chlorpromazine and hyperbaric oxygen.[14] In spite of favorable results the procedure is not used because of the complexity and difficulty of the technique. On the other hand, perfusion method also supplies oxygen to the graft but is not used for small bowel preservation because this method incurs the risk of greater vascular injury.[16] After all, simple cold storage is the most practical method. Based on these results, we have developed a two-layer (UW／PFC) cold storage method which supplies sufficient oxygen and made it possible to prolong canine pancreas preservation time up to 96hr.[6] We have also reported a successful 48hr heart preservation with a cavitary two-layer (UW／PFC) cold storage method in rats.[12] In this study, we applied the cavitary two-layer method to rat small bowel preservation and obtained successful results in heterotopic and segmental transplant model. The small-intestinal mucosa, more than any other structural layer of the small bowel, is highly sensitive to ischemia but it also has a great regenerative potential.[19] Recovery of the normal structure of the mucosa occurs within 2-7days after significant ischemia in the rat.[4,17,26] However, poor preservation causes a graft necrosis. Acute graft necrosis results in immediate recipient death due to massive intraluminal hemorrhage. Therefore the graft survival rate is thought to be related to acute graft failure due to inadequate preservation and we assessed the histology of the graft at 7days after transplantation. Surviving animals had consistently the normal architecture of the small-intestinal mucosa and there was no surviving animal with bleeding or necrosis of graft. Animals which had bleeding or necrosis of graft died within 3 days and there was no died animals with normal architecture of the small-intestinal mucosa. Seven day animal survival

and the histologic evaluation of the graft at 7 days after transplantation indicates whether a particular method of preservation was effective. In this study, we also evaluated the histology of the graft immediately after preservation. The longer the preservation period was, the severer the ischemic damage was. Cell damages in groups 1B and 3B were greater than groups 1A and 3A. However, no differencies were found between the two-layer storage group (group 1) and simple storage in UW group (group 3) in the same preservation time (1A and 3A, 1B and 3B), although graft survival and the histology at 7 days after transplantation is completely different among these groups (Fig.3A-3D). It is clear that the histologic findings of the small bowel graft immediately after preservation do not predict the graft or animal survival. By the simple cold storage method with UW solution, 48hr-preservation was unsuccessful. In contrary, the cavitary two-layer method allowed for 48hr-preservation. Without oxygenation it did not allow more than 24hr-preservation. It is reasonable to suppose that oxygenation of the small bowel contributes to an extended period of preserved graft viability. As it was clarified that oxygenation of the pancreas graft during cold storage was due to direct diffusion of dissolved oxygen in PFC through the undersurface of the graft contacted with PFC,[10] we modified the two-layer cold storage method for small bowel preservation and kept the whole graft into O_2-saturated PFC and preservation solution inside the lumen. As we made it clear that the delivery of oxygen to the heart during preservation by the cavitary two-layer method is mainly due to the direct diffusion of dissolved oxygen in PFC through the pericardium of the heart,[12] it seems reasonable to think that oxygen is delivered to the small bowel through the serosa contacted with O_2-saturated PFC during preservation. Belzer et al. reported that adenosine should be omitted in the composition of preservation solutions, since adenosine, as a precursor of hypoxanthine , might increase xanthine oxidase activity with subsequent generation of superoxide anions.[3] But we preveously reported that provision of adenosine to the graft stimulates ATP synthesis and improves the viability of the canine pancreas graft during preservation by the two-layer method.[5] Therefore we used the UW as a preservation solution for small bowel. Preliminary experiments have shown that ATP tissue levels of the graft during preservation by the cavitary two-layer method with oxygen bubbling is significantly higher compared with the values without oxygen bubbling (manuscript in

preparation). The enterocytes of the small bowel preferentially use glutamine as a respiratory fuel and as an essential source of carbon and nitrogen for synthesis of nucleic acid and protein[21,27] which are essential for restoration and maintenance of the intestinal mucosa. Other major fuels used by the small bowel are the ketone bodies, β - hydroxybutyrate and acetoacetate.[20] Further studies are needed to determine if these substances should be contained in preservation solution as energy substrates during preservation by the cavitary two-layer method. As the small bowel is exposed to the other environment including microorganisms and toxins and intestinal mucosa may be damaged by contact with pancreatic and other proteolytic enzymes during storage,[2,18] we cleaned the lumen of harvested small bowel using saline with 0.5% gentamicin.

This study demonstrates that the oxygenation of the small bowel during preservation by the cavitary two-layer method using UW makes it possible to extend preservation time up to 48hrs in heterotopic rat segmental small bowel transplant model. Although we must assess this method concerning graft survival and physiological function in orthotopic small bowel transplant model, this method is simple and reliable, and holds promise for clinical small bowel transplantation.

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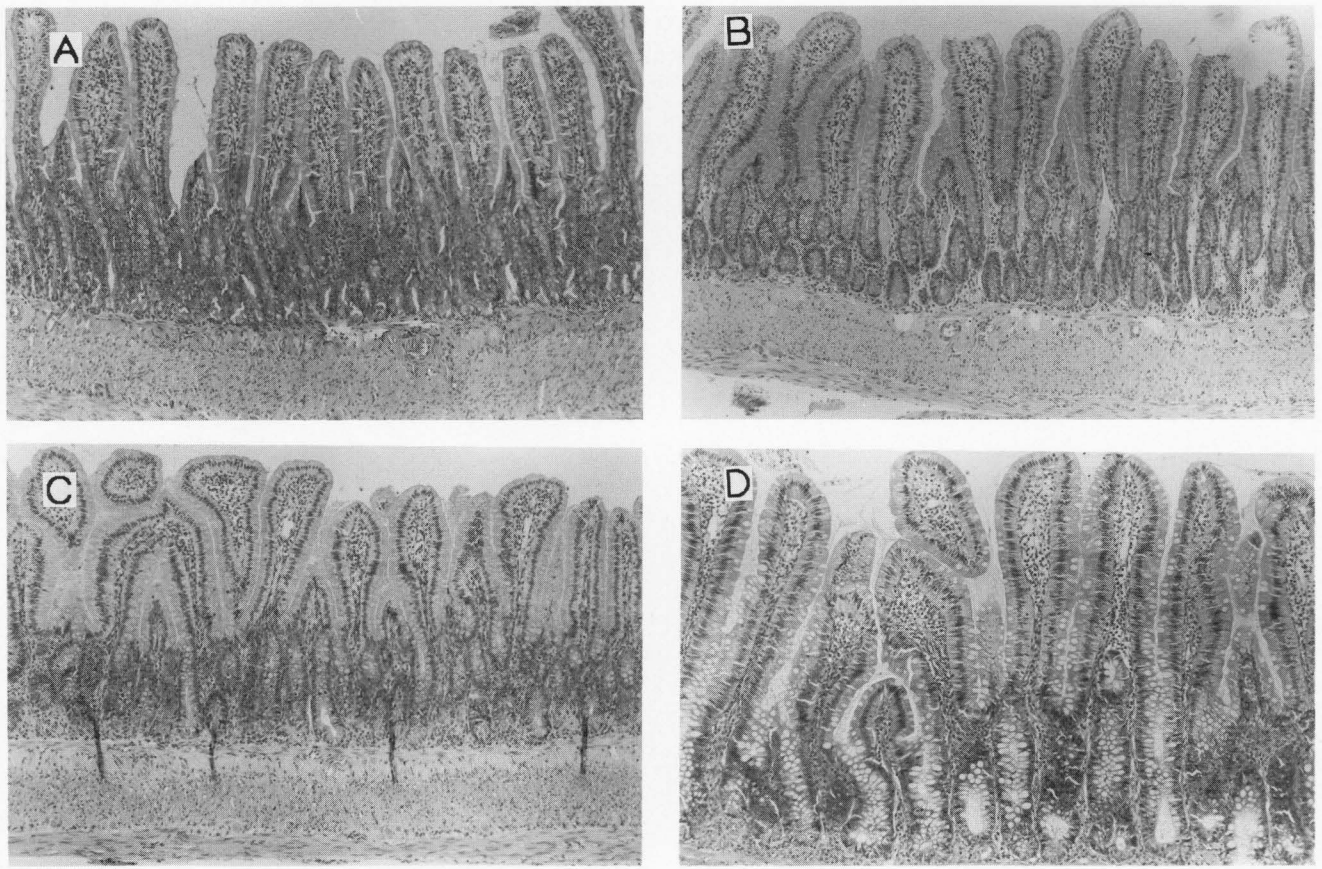

Fig. 2. Photomicrographs of the grafts taken at 7days after transplantation. (A) control(group 4), (B) simple storage method for 24hrs (group 3A), (C) cavitary two-layer method with O_2 bubbling for 24hrs (group 1A), (D) cavitary two-layer method with O_2 bubbling for 48hrs (group 1B). (H&E; X200)

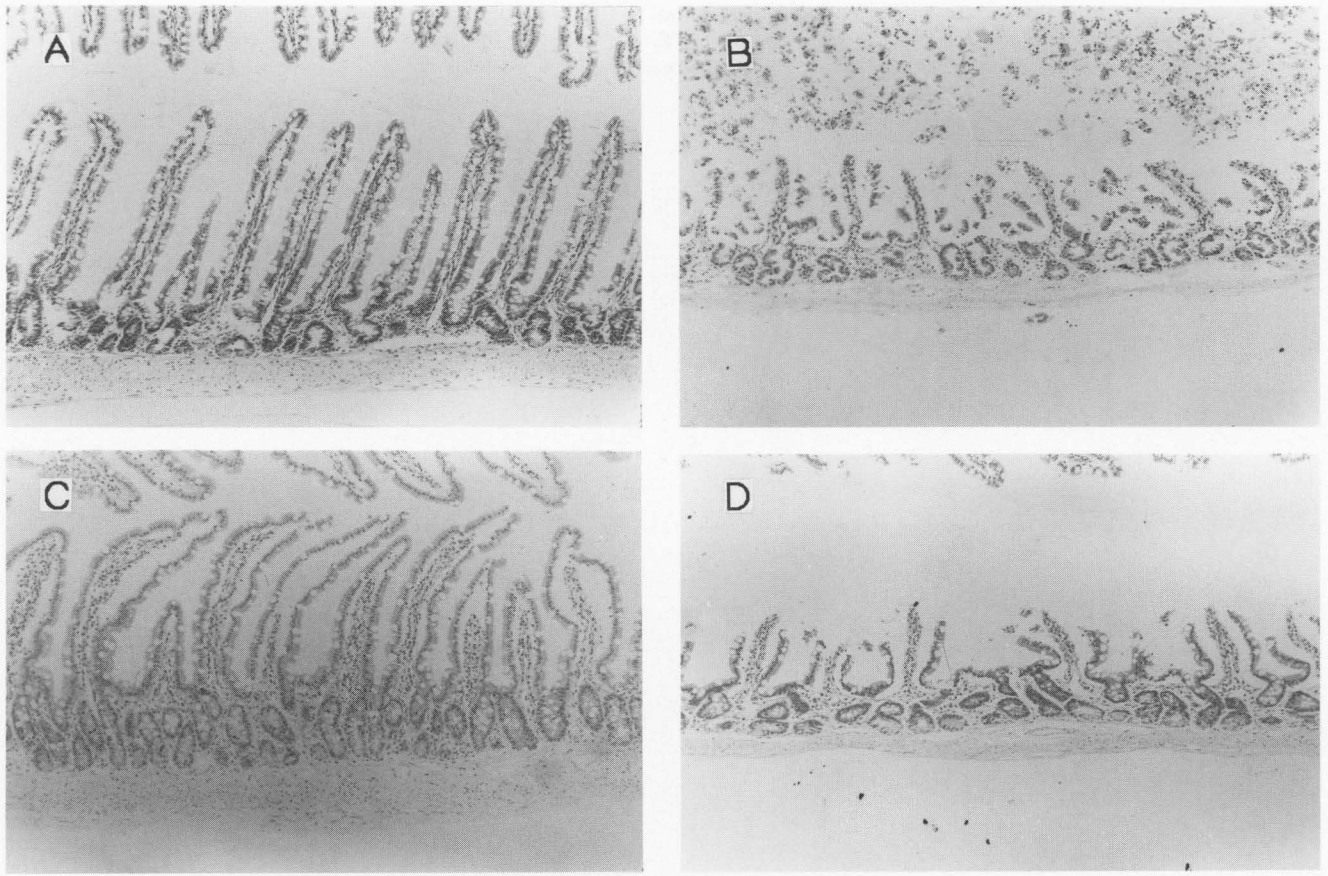

Fig. 3. Photomicrographs of the grafts taken immediately after preservation. (A) simple storage method for 24hrs (group 3A), (B) simple storage method for 48hrs (group 3B), (C) cavitary two-layer method with O_2 bubbling for 24hrs (group 1A), (D) cavitary two-layer method with O_2 bubbling for 48hrs (group 1B). (H&E; X200)