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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:
A01N 1/02	A1	WO 94/21116
		(43) International Publication Date: 29 September 1994 (29.09.94)

(21) International Application Number: PCT/US94/02831

(22) International Filing Date: 16 March 1994 (16.03.94)

(30) Priority Data:
08/033,629 16 March 1993 (16.03.93) US

(60) Parent Application or Grant
(63) Related by Continuation
US 08/033,629 (CIP)
Filed on 16 March 1993 (16.03.93)

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PRESERVATION SOLUTION AND METHOD FOR WARM ORGAN PRESERVATION

(57) Abstract

The present invention is directed to a new hyperosmolar preservation solution useful for the initial flushing and for the storage of organs intended for transplantation using a warm preservation technology, between 180 °C and 35 °C. Among the components of the preservation solution are a basal medium comprising impermeant, mucopolysaccharide, and a high magnesium content, and an emulsified liquid fluorocarbon.

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PRESERVATION SOLUTION AND METHOD FOR WARM ORGAN PRESERVATION

Field of the Invention

The present invention relates to a solution for use in preserving organs and tissues and a method for allograft preservation. More specifically, the invention comprises a method for the preservation of organs without hypothermia using a perfusate supplemented with an emulsified liquid fluorocarbon.

Background of the Invention

In vitro organ preservation was originally described by Carrel and Lindberg in 1938. Since their original studies, many researchers have attempted to preserve organs. Over the subsequent years, the longest experimental preservation times achieved have been approximately five to seven days for kidneys. When one reviews the literature on organ preservation, there are almost no reports close to achieving the three week experimental preservation of Carrel. This may be due to the fact that organ preservation studies embarked upon a path utilizing hypothermic conditions very early on. In 1937, Bickford and Winton noted that hypothermia prolonged the duration of tissue survival since hypothermia reduces the metabolic need of an organ. At 25°C, there was a 25% reduction of blood flow in the kidney and the creatinine clearance was reduced by 20%. Below 18°C hypothermia inhibits the tubular activity of the kidney. At 4°C, the utilization of oxygen is approximately 5% of that at normothermia.

A direct relationship between oxygen requirements and temperature exists. In fact, hypothermia may exert a greater depression of oxidative metabolism in the kidney than in the body as a whole and this may help to explain the success in preserving kidney relative to other organs. A major portion of the oxygen consumed by the kidney is utilized for the process of active sodium reabsorption and sodium reabsorption is by far the most important of all the tubular transport processes. Hypothermic storage, however, is not benign. It produces vasospasm and subsequent edema in an allograft.

Preserved organs experience glomerular endothelial cell swelling and loss of vascular integrity along with tubular necrosis. These phenomena can be attributed to the hypothermia. Hypothermia inhibits Na/K dependent ATPase and results in the loss of the cell volume regulating capacity. The loss of volume regulation is what causes the cellular swelling and damage. An ample supply of oxygen actively diminishes the amount of this swelling. The most successful early organ preservation studies employed either supplementation with hemoglobin or a pump-lung system. Without adequate oxygen delivery, anoxia leads to disintegration of the smaller vessels after several hours of perfusion.

Accordingly, supplying adequate oxygen delivery to the organ has been a major obstacle to successful organ preservation. The inability to supply oxygen lead to the reliance on hypothermia. And yet, the hypothermia itself may represent the rate-limiting factor in organ preservation. The lack of oxygen and the subsequent depletion of ATP stores mean that anaerobic glycolysis is the principal source of energy under traditional preservation conditions. Therefore, glycolysis is the only source of ATP available to anoxic cells.

It is well known that hypothermic conditions eliminate the effective utilization of O_2 by tissues. At normal physiologic temperatures, the phospholipids making up the cell membranes are highly fluid. Under hypothermic conditions, the lipid bi-layer experiences a phase-change and becomes gel-like, with greatly reduced fluidity. The essentially frozen lipid in the cell membranes negates the utilization of O_2, even in the presence of a high O_2-tension. The metabolic consequence is glycolysis, which is analogous to the state of anoxia. The hypothermic conditions utilized in organ preservation probably represent the reason why previous studies raising the O_2-tension did not uniformly demonstrate the benefit of increased oxygenation of tissues.

It has been estimated that approximately one third of the

energy metabolism of cells is expended to maintain the sodium pump and to preserve cell volume. The lack of molecular oxygen for oxidative phosphorylation which occurs in ischemia leads to the accumulation of NADH and the depletion of ATP stores within the mitochondria. The subsequent loss of nucleosides is probably a very important factor in the failure of tissues subjected to warm ischemia and prolonged periods of cold ischemia to regenerate ATP after restoration of the blood supply.

The development of warm preservation technology would present the opportunity to support near normal metabolic activity. In order to support the increased metabolic rate, an increased O_2-tension will be required for respiration. Extending the period of organ preservation *in vitro* further cannot be achieved without increasing the metabolic activity suppressed by the hypothermia and in turn supplying adequate oxygen and metabolite delivery to support this basal metabolism.

Currently, kidney transplantation is largely dependent upon the availability of organs retrieved from heartbeating cadaver donors. While awaiting transplantation, kidneys must be stored in a fashion that will result in the restoration of normal, immediate function. Therefore, the clinical preservation of organs is much more limited than the experimental models; approximately 48 hours for kidneys, 18 hours for livers and 4 hours for hearts. There are two methods of storage for kidney - preservation by continuous hypothermic perfusion and simply hypothermic storage. While a variety of perfusates have been utilized clinically, these two methods of kidney storage have remained substantially unchanged for the past 20 years. Viaspan™, manufactured and marketed by DuPont, represents state-of-the-art organ preservation. ViaSpan™, originally developed as the UW solution, provides for optimized organ preservation under hypothermic conditions. While ViaSpan™ minimizes the edema and vasospasm normally encountered during hypothermic storage, it does not provide for the utilization of an expanded donor

pool. This is due to the fact that an allograft marginally damaged by warm ischemia cannot tolerate further damage mediated by the hypothermia. Until a mechanism for organ preservation is developed which will provide for the utilization of an expanded donor pool, the severe shortage of organs in the U.S. cannot be met.

ViaSpan™ contains components which prevent hypothermic induced tissue edema, metabolites which facilitate organ function upon transplantation, anti-oxidants, membrane stabilizers, colloids, ions and salts. The formulation of this perfusate is designed to preserve the kidney allografts by hypothermic induced depression of metabolism. Yet, the clinical preservation times have not been extended. There is a need for the development of more efficient preservation technology. Better quality preservation will also provide the opportunity to expand the donor pool. Allografts with marginal function, secondary to warm ischemia, could theoretically be utilized because further damage via hypothermic preservation would be eliminated.

The current demand for organs cannot be met unless new sources of organs can be developed. And yet, the donor pool cannot be substantially expanded with existing technology because the rapid cooling of organs and the use of high potassium solutions eliminate the possibility of instituting any concordant, life-saving measures. A large and as yet untapped source of organs for transplantation are accident victims who succumb at the accident site and those who have short post-trauma survival times. These accident victims are not used as organ donors because of the ischemic damage. A period of warm ischemia greater than 60 minutes leads to the phenomenon of no-reflow, which is the failure of circulation to return to the organ. If a suitable means of organ preservation could be developed which minimizes, or even repairs, the damage due to warm ischemia, the shortage of organs could be largely alleviated. The development of warm preservation technology would present the unique opportunity of supporting both organ-salvage and life-saving protocols

simultaneously. As stated above, with traditional techniques, patient life-saving measures and organ saving measures are mutually exclusive. The development of warm preservation technology would present the possibility for *in vivo* flushing which would satisfy patient survival and organ salvage protocols. By conservative estimates, this untapped pool of organ donors could provide approximately 10 times the number of organs currently being harvested in the U.S. The number of patients being maintained on hemodialysis has almost doubled in the last six years. Therefore, there is tremendous pressure in the U.S. to open new avenues of procuring transplantable organs. This building public pressure being placed on the medical community is reflected by the recent attempts at xenografting and the discussions pertaining to the sale of organs.

There is therefore an urgent need for a preservation solution useful for initial organ flushing and as a perfusate for *in vitro* preservation of organs for transplantation using a warm preservation technology which will minimize, or even repair, the damage caused by warm ischemia, and which supports the organ near normal metabolic rate. A desirable feature of using such a solution is that organ preservation *in vitro* may be extended further by increasing metabolic activity, suppressed by hypothermia, by supplying adequate oxygen and metabolite delivery to support this basal metabolism.

Fluorocarbons are fluorine substituted hydrocarbons that have been used in medical applications as imaging agents and as blood substitutes. U.S. Patent No. 3,975,512 to Long discloses fluorocarbons, including brominated fluorocarbons, for use as a contrast enhancement medium in radiological imaging. Brominated fluorocarbons and other fluorocarbons are known to be safe, biocompatible substances when appropriately used in medical applications.

It is additionally known that oxygen, and gases in general, are highly soluble in some fluorocarbons. This characteristic has permitted investigators to develop emulsified fluorocarbons as blood substitutes.

Injectable fluorocarbon emulsions act as a solvent for oxygen. They dissolve oxygen at higher tensions and release this oxygen as the partial pressure decreases. Carbon dioxide is handled in a similar manner. Oxygenation of the fluorocarbon when used intravascularly occurs naturally through the lungs. For other applications, the fluorocarbon can be oxygenated prior to use.

Thus, fluorocarbons represent a safe, biocompatible source of oxygen which can support basal metabolism. There is a need for further development of fluorocarbon technology for medical applications.

Summary of the Invention

In accordance with one aspect of the present invention, there is provided a method for preserving an organ prior to transplantation, comprising the steps of flushing the organ with a preservation medium comprising an aqueous solution having an osmolarity of from about 300 to about 1000 mOsM and emulsified liquid fluorocarbon, wherein said fluorocarbon comprises between about 1% and 50% v/v of said preservation medium. Thereafter, the organ is stored at a temperature between about 18°C and about 35°C for at least one hour. The flushing step can be performed *in vivo*, or alternatively, the flushing step and the storing step can be performed while said organ remains *in situ* or *in vitro*. The method of the present invention can also include the step of continuously perfusing the organ with the preservation medium during the storing step. In this method of the present invention, the aqueous solution preferably comprises buffered basal medium, impermeant, mucopolysaccharide, and magnesium, and further preferably comprises buffered basal medium supplemented with components such as amino acids, ions, physiological salts, colloids, serum proteins, carbohydrates, lipid, attachment factors, and growth factors, or functional equivalents thereof, and preferably has an osmolarity of from about 400 to about 900 mOsM. More preferably, the osmolarity is from about 350 to about 380 mOsM. The preservation solution used in the method of the present invention also preferably contains a

fluorocarbon which comprises between about 5% to about 40% v/v of the preservation medium.

In accordance with another aspect of the present invention, there is provided a hyperosmolar preservation solution which supports the preservation of organs without the need for hypothermia. This solution comprises an aqueous solution having an osmolarity of from about 300 to about 1000 mOsM and emulsified liquid fluorocarbon, wherein the fluorocarbon comprises between about 1% and 50% v/v of said preservation solution. The aqueous solution preferably comprises buffered basal medium, impermeant, mucopolysaccharide, and magnesium, and is preferably supplemented with components such as amino acids, ions, physiological salts, colloids, serum proteins, carbohydrates, lipid, attachment factors, and growth factors, or functional equivalents thereof. The aqueous solution component of the preservation solution of the present invention preferably has an osmolarity of from about 400 to about 900 mOsM, and more preferably, an osmolarity of from about 350 to about 380 mOsM. The fluorocarbon component of the preservation solution of the present invention preferably comprises between about 5% to about 40% v/v of said preservation medium. The hyperosmolar preservation solution, when used for initial flushing of an organ, preferably includes mannitol, at a concentration of from about 1 to about 100g/L. Preferably, the impermeant used in the preservation solution of the present invention is cyclodextrin, at a concentration of from about 0.1 to about 1g/L, and the mucopolysaccharide is chondroitin sulfate, at a concentration of from about 1 to about 50mg/L. The magnesium added to the preservation solution of the present invention is preferably in the form of magnesium sulfate, present at a concentration of from about 0.08 to about 4g/L.

Brief Description of the Figures

FIGURE 1 graphically represents the PO_2 in kidneys flushed with basal preservation solution.

FIGURE 2 graphically represents the PO_2 in kidneys flushed with basal preservation solution supplemented with an

emulsified liquid fluorocarbon.

Detailed Description of the Invention

The present invention is directed to a new hyperosmolar aqueous solution particularly useful as a preservation solution for the initial organ flushing, and/or as a perfusate for storage of organs using a warm preservation technology (18-35°C) without the need for extreme hypothermia. The preservation solution of the present invention comprises a hyperosmolar aqueous solution, between about 400 and 900 mOsM (or optionally between about 300 and 400 mOsM), and an emulsified liquid fluorocarbon, wherein the fluorocarbon comprises between about 1% and about 50% v/v of the preservation medium. The solution may optionally have components designed to support the nutritional and metabolic needs of the vascular endothelium within a graft at temperatures between about 18°C and 35°C, thereby maintaining the integrity of the vasculature and subsequently the normal permeability of the organ. One embodiment of the aqueous solution comprises a buffered basal medium, impermeant, mucopolysaccharide, and magnesium. Among the additional components may be amino acids, ions, physiologic salts, serum, serum proteins, sugars, lipids, attachment factors, and growth factors. This perfusate is able to preserve organs without concomitant extreme hypothermia.

While the studies described herein relate to the preservation of renal allografts, the application of the solution using a warm preservation technique relates to the preservation of other organs as well. While some of the components of the solution of the present invention are similar to those of other known tissue culture media, and of other known preservation solutions for use in organ transplantation with extreme hypothermia, the solution of the present invention was specifically designed to potentiate the simultaneous growth of microvessel and large vessel endothelial cells, to support the integrity of vascular endothelium within a graft, and to support more normal permeability and metabolism without extreme hypothermia.

The preservation solution of the present invention may advantageously employ a cell culture-like basal medium, to which is added a variety of supplements including an emulsified liquid fluorocarbon. The enhanced ability of the solution of the present invention to serve as a preservation solution for organs for transplantation using a warm preservation technology may be facilitated by supplementation with serum albumin as a source of protein and colloid; trace elements to potentiate viability and cellular function; pyruvate and adenosine for oxidative phosphorylation support; transferrin as an attachment factor; insulin and sugars for metabolic support; mannitol and glutathione to scavenge toxic free radicals as well as a source of impermeant and to support a brisk diuresis; cyclodextrin as a source of impermeant, scavenger, and potentiator of cell attachment factors; a very high Mg^{++} concentration for microvessel metabolism support; mucopolysaccharides, comprising primarily chondroitin sulfates, for growth factor potentiation and hemostasis; ENDO GRO™ as a source of colloid, impermeant and growth promoter; and an emulsified liquid fluorocarbon to supply the necessary oxygen.

ENDO GRO™ is a bovine retinal-derived growth factor which is effective in promoting cell proliferation with low serum concentration, and is available from VecTec, Inc., Schenectady, New York. It is an anionic heparin binding glycoprotein with a molecular weight of 18,000 and an isoelectric point of 4.5.

The osmolarity of the preservation solution of the present invention should preferable range from about 300 to about 1000 mOsM. More preferably, the osmolarity should range from about 400 to about 900 mOsM. In an alternative embodiment, the osmolarity should range from about 300 mOsM to about 380 mOsM. The osmolarity of the present invention is provided by the albumin, glucose, ENDO GRO™, transferrin and cyclodextrin.

The preservation solution of the present invention has been found to preserve organs without extreme hypothermia, and

does not present the common problems encountered with cold storage perfusates, namely edema, vasospasm, depletion of ATP stores, shutdown of ion pumps, glycolysis, and the generation of cold-induced toxic free radical intermediates. The preservation solution of the present invention may provide for more efficacious preservation thereby presenting the potential to utilize nonheartbeating cadaver donors, thus expanding the potential donor pool.

In addition, the present invention has direct applications for the preservation of the vasculature in any form of trauma, including infarction or aneurysm, involving ischemia or hemorrhage. Likewise, localized preservation of isolated anatomy is possible. The technology of the present invention can be used to preserve high oxygen-consuming organs such as the eye in cases of retinopathy or macular degeneration. The preservation solution and method of use of the present invention can also be used in cases of controlled ischemia during surgical procedures, as well as in the areas of limb salvage and organ repair.

The basal solution of the present invention can be prepared according to the constituent ranges set forth in Table 1 below. An emulsified liquid fluorocarbon is then added to this basal solution to produce a solution useful in preserving organs for transplantation using warm preservation technology.

TABLE 1

COMPOSITION OF THE BASAL PRESERVATION SOLUTIONS OF THE PRESENT INVENTION

	Basal Medium Ranges	Formulation 1	Formulation 2
DL-Alanine	.001-5g/L	.06g/L	.12g/L
L-Arginine HCl	.001-5g/L	.07g/L	.14g/L
DL-Aspartic Acid	.001-5g/L	.06g/L	.12g/L
L-Cysteine HCL•H_2O	.0001-1g/L	.00011g/L	.00022g/L
L-Cystine 2HCl	.001-5g/L	.026g/L	.052g/L
DL-Glutamic Acid	.001-5g/L	.1336g/L	.2672g/L
L-Glutamine	.001-5g/L	.10g/L	.2g/L
Glycine	.001-5g/L	.05g/L	.1g/L
L-Histidine HCl•H_2O	.001-5g/L	.02188g/L	.04376g/L
L-Hydroxyproline	.001-5g/L	.01g/L	.02g/L
DL-Isoleucine	.001-5g/L	.04g/L	.08g/L
DL-Leucine	.01-5g/L	.12g/L	.24g/L
L-Lysine HCl	.001-5g/L	.07g/L	.14g/L
DL-Methionine	.001-5g/L	.03g/L	.06g/L
DL-Phenylalanine	.001-5g/L	.05g/L	.10g/L
L-Proline	.001-5g/L	.04g/L	.08g/L
DL-Serine	.001-5g/L	.05g/L	.10g/L
DL-Threonine	.001-5g/L	.06g/L	.12g/L
DL-Tryptophan	.001-5g/L	.02g/L	.04g/L
L-Tyrosine•2Na	.001-5g/L	.05766g/L	.11532g/L
DL-Valine	.001-5g/L	.05g/L	.10g/L
Adenine Hemisulfate	.001-5g/L	.01g/L	.02g/L
Adenosine Triphosphate•2Na	.0001-1g/L	.001g/L	.002g/L
Adenylic Acid	.00001-1g/L	.0002g/L	.0004g/L
Alpha Tocopherol Phosphate•2Na	.000001-1g/L	.00001g/L	.00002g/L
Ascorbic Acid	.000001-1g/L	.00005g/L	.0001g/L
d-Biotin	.000001-1g/L	.00001g/L	.00002g/L
Calciferol	.00001-1g/L	.0001g/L	.0002g/L
Cholesterol	.00001-1g/L	.0012g/L	.0024g/L
Choline Chloride	.00001-1g/L	.0005g/L	.001g/L
Deoxyribose	.00001-1g/L	.0005g/L	.001g/L
Folic Acid	.000001-1g/L	.0001g/L	.0002g/L
Glutathione (Reduced)	.000001-1g/L	.00005g/L	.0001g/L
Guanine HCl	.00001-1g/L	.0003g/L	.0006g/L
Hypoxanthine	.00001-1g/L	.0003g/L	.0006g/L
Menadione (Na Bisulfite)	.000001-1g/L	.000016g/L	.00003g/L
Myo-Inositol	.000001-1g/L	.00005g/L	.0001g/L
Niacinamide	.000001-1g/L	.000025g/L	.00005g/L
Nicotinic Acid	.000001-1g/L	.000025g/L	.00005g/L
PABA	.000001-1g/L	.00005g/L	.0001g/L
D-Pantothenic Acid Ca	.000001-1g/L	.00001g/L	.00002g/L
Polyoxyethylene-sorbitan Monooleate	.001-1g/L	.02g/L	.04g/L

Pyridoxal HCl	.000001-1g/L	.000025g/L	.00005g/L
Pyridoxine HCl	.000001-1g/L	.000025g/L	.00005g/L
Retinol Acetate	.00001-1g/L	.00014g/L	.00028g/L
Riboflavin	.000001-1g/L	.00001g/L	.00002g/L
Ribose	.00001-1g/L	.0005g/L	.001g/L
Thiamine HCl	.C00001-1g/L	.00001g/L	.00002g/L
Thymine	.00001-1g/L	.0003g/L	.0006g/L
Uracil	.00001-1g/L	.0003g/L	.0006g/L
Xanthine•Na	.00001-1g/L	.000344g/L	.00069g/L
Calcium chloride•$2H_2O$	.01-5g/L	.265g/L	.53g/L
Ferric Nitrate•$9H_2O$	.00001-1g/L	.00072g/L	.00144g/L
Magnesium sulfate (anhydrous)	.001-5g/L	1.30117g/L	2.60234g/L
Potassium chloride	.01-5g/L	.40g/L	.8g/L
Sodium Acetate (anhydrous)	.001-5g/L	.05g/L	.1g/L
Sodium Chloride	1-20g/L	6.8g/L	6.8g/L
Sodium Phosphate Monobasic (anhydrous)	.01-5g/L	.122g/L	.244g/L
Glucose	.1-5g/L	1.0g/L	2.0g/L
Phenol Red Na	.001-5g/L	.0213g/L	.0426g/L
$FeSO_4$	.000278g/L		
$CuSO_4$		.0000025g/L	.0000025g/L
Insulin		.005g/L	.01g/L
Bovine serum albumin (BSA)		5g/L	10g/L
Manganese Sulfate		.0000014g/L	.0000014g/L
$NaHCO_3$		2.2g/L	2.2g/L
$NaSeSO_3$		.000005g/L	.000005g/L
NH_4VO_3		.000058g/L	.000058g/L
$NiCl_2$		.000012g/L	.000012g/L
Putrescine		.000044g/L	.000044g/L
Pyruvate		.11g/L	.22g/L
Thymidine		.00007g/L	.00007g/L
Transferrin		.05g/L	.1g/L
Thyronine		.0000002g/L	.000002g/L
Vitamin B_{12}		.00002g/L	.00002g/L
Amphotericin B		.00025g/L	.0005g/L
Penicillin - units		100,000U	200,000U
Streptomycin		.10g/L	.20g/L
Mercaptoethanol		..0007ml	.0007ml
Fetal bovine serum (FBS)		10ml	100ml
Impermeant (Cyclodextrin)		.25g/L	.5g/L
Mucopolysaccharide (chondroitin sulfate B)		.002g/L	.004g/L
ENDO GRO™		.09g/L	.20g/L
heparin		.18g/L	.18g/L
mannitol			10g*

* - for flush only

While it is contemplated that the various components of the basal preservation solution of the present invention may be mixed in a liter of distilled water to produce the

formulation, there may exist various commercial preparations that contain many of the components, in the desired constituent ranges, of the basal medium, and that those components deficient in the particular commercial preparation may be added to that preparation to produce the basal formulation of the present invention.

In addition to this basal medium, the preservation solution of the present invention also includes an emulsified liquid fluorocarbon. This fluorocarbon emulsion comprises from approximately 1% to 50% of the preservation solution (v/v). More preferably, the emulsified fluorocarbon comprises from about 5% to about 40% of the total solution (v/v). Fluorocarbon compounds useful in this invention are generally able to promote gas exchange, and most of these fluorocarbons readily dissolve oxygen and carbon dioxide. As is known in the art, emulsions having a particle size significantly greater than 0.4 microns tend to occlude small vessels and to collect too rapidly in the liver, spleen and other organs, enlarging them and endangering their function. Thus, the fluorocarbon emulsion of the present invention preferably has a particle size of less than about 0.5 microns.

There are a number of fluorocarbons that are contemplated for medical use. These fluorocarbons include bis(F-alkyl) ethanes such as $C_4F_9CH=CH_4CF_9$ (sometimes designated "F-44E"), i-$C_3F_9CH=CHC_6F_{13}$ ("F-i36E"), and $C_6F_{13}CH=CHC_6F_{13}$ ("F-66E"), cyclic fluorocarbons, such as C10F18 ("F-decalin," "perfluorodecalin" or "FDC"), F-adamantane ("FA"), F-methyladamantane ("FMA"), F-1,3-dimethyladamantane ("FDMA"), F-di-or F-trimethylbicyclo[3,3,1]nonane ("nonane"); perfluorinated amines, such as F-tripropylamine ("FTPA") and F-tri-butylamine ("FTBA"), F-4-methyloctahydroquinolizine ("FMOQ"), F-n-methyl-decahydroisoquinoline ("FMIQ"), F-n-methyldecahydroquinoline ("FHQ"), F-n-cyclohexylpurrolidine ("FCHP") and F-2-butyltetrahydrofuran ("FC-75" or "RM101").

Other fluorocarbons include brominated perfluorocarbons, such as 1-bromo-heptadecafluoro-octane ($C_8F_{17}Br$, sometimes designated perfluorooctylbromide or "PFOB"), 1-bromopenta-

decafluoroheptane ($C_7F_{15}Br$), and 1-bromotridecafluorohexane ($C_6F_{13}Br$, sometimes known as perfluorohexylbromide or "PFHB"). Other brominated fluorocarbons and fluorocarbon emulsions suitable for use in the present invention can be of the type described in U.S. Patent No. 3,975,512 to Long, which also describes methods of preparing fluorocarbon emulsions. Also contemplated are fluorocarbons having nonfluorine substituents, such as perfluorooctyl chloride, perfluorooctyl hydride, and similar compounds having different numbers of carbon atoms.

Additional fluorocarbons contemplated in accordance with this invention include perfluoroalkylated ethers or polyethers, such as $(CF_3)_2CFO(CF_2CF_2)_2OCF(CF_3)_2$, $(CF_3)_2CFO-(CF_2CF_2)_3OCF(CF_3)$, $(CF_3)CFO(CF_2CF_2)F$, $(CF_3)_2CFO(CF_2CF_2)_2F$, $(C_6F_{13})_2O$. Further, fluorocarbon-hydrocarbon compounds, such as, for example compounds having the general formula $C_nF_{2n+1}-C_{n'}F_{2n'+1}$, $C_nF_{2n+1}OC_{n'}F_{2n'+1}$, or $C_nF_{2n+1}CF=CHC_{n'}F_{2n'+1}$, where n and n' are the same or different and are from about 1 to about 10 (so long as the compound is a liquid at room temperature). Such compounds, for example, include $C_8F_{17}C_2H_5$ and $C_6F_{13}CH=CHC_6H_{13}$. It will be appreciated that esters, thioethers, and other variously modified mixed fluorocarbon-hydrocarbon compounds are also encompassed within the broad definition of "fluorocarbon" materials suitable for use in the present invention. Mixtures of fluorocarbons are also contemplated. Additional "fluorocarbons" not listed here, but having those properties described in this disclosure are additionally contemplated.

Preparation of fluorocarbon emulsions is well known and is described, for example, in US Patent 5,080,885. Such emulsions are also available from Alpha Therapeutics (Boston, MA) under the trademark FLUOSOL and Alliance Pharmaceutical Corp. (San Diego, CA) under the trademark OXYGENT.

Particularly preferred fluorocarbon compounds for use in the present invention are the brominated perfluorocarbons. Perfluorooctylbromide, or PFOB, is particularly preferred. An emulsified liquid fluorocarbon is added to the basal solution

to produce the preservation solution of the present invention. Such an emulsion can be prepared using the following components: PFOB, EYP, NaH_2PO_4, Na_2HPO_4, $CaNa_2EDTA$, d-α Tocopherol, and NaCl. A particularly preferred formulation is set forth in Table 2 below.

TABLE 2

Component	% w/v
PFOB	90.0
EYP	4.0
NaH_2PO_4	0.052
Na_2HPO_4	0.355
$CaNa_2EDTA$	0.02
d-α Tocopherol	0.002
NaCl	0.30

Another particularly preferred embodiment of the fluorocarbon emulsion of the present invention comprises the following:

perfluorocarbon: about 20-100% w/v
egg yolk phospholipid: about 2-10% w/v
sodium phosphate buffer: about 0.25-1.5% w/v
particle size: median range of 0.1-0.5 micron
pH: 6-7.5

The following examples further illustrate the use of the liquid fluorocarbon emulsion preservation solution of the present invention as a perfusate for organ preservation using a warm preservation technology. Although the Examples described herein disclose the use of a perfluorooctylbromide (PFOB) emulsion, the preferred fluorocarbon emulsion, other oxygen carrying fluorocarbons in an emulsified form may be used as well.

EXAMPLE 1

The Effect of Supplementation with PFOB

To determine the effect of the supplementation of the basal medium with a liquid fluorocarbon emulsion, two experimental approaches were taken. The first approach employed simple static preservation at 25°C and the second

approach used pulsatile preservation at 32°C. All experiments were performed in duplicate and the histology was performed blindly.

A. Static Storage

Canine kidneys were dissected using a midline incision. The renal arteries were identified and the kidneys were isolated with the vasculature intact. The renal arteries were then cannulated and the kidneys flushed of blood with approximately 250cc of the basal medium solution. One set of kidneys was flushed with basal solution supplemented with 20% PFOB v/v (as a 90% emulsion) and one set was flushed with basal solution without the PFOB supplementation. Using this method, the warm ischemia time was less than five minutes. The flushed kidneys were then stored statically in perfusate, one containing no further supplementation and one supplemented with 20% PFOB v/v (as a 90% emulsion). The kidneys were stored at 25°C for 24 hours. After storage, the kidneys were fixed in buffered formalin and evaluated histologically.

Results

The glomeruli were normal in all kidneys. The tubules were normal in appearance in all kidneys. A few vacuoles appeared in the straight proximal tubules in kidneys stored in basal solution without PFOB supplementation. There was focal mononuclear cell infiltrate in the medulla in those kidneys stored in solution with PFOB supplementation.

B. Pulsatile Preservation

The canine kidneys were harvested as described above. After cannulation, the renal arteries were flushed with approximately 250cc of the basal flushing solution. One set of kidneys was flushed with a solution supplemented with 20% PFOB v/v (as a 90% emulsion) and the other set was flushed with a solution containing no PFOB. After flushing, the kidneys were placed on a Waters MOX-100 preservation system and pumped for approximately ten hours at 32°C. A pulse rate of 60/minute was maintained with systolic pressures below 70mmHg.

Results

a. Flow Characteristics

Overall flow dynamics during preservation were quite similar in kidneys perfused with basal solution with or without PFOB supplementation. The regulation of pressure, pH and vascular resistance were equivalent. PFOB supplementation does, however, affect flow rates. Without PFOB supplementation, the flow rates ranged from approximately 10-16 seconds to clear 20cc of perfusate through the renal vasculature. When PFOB (20%) is added to the perfusate, the flow rates decreased slightly to the range of approximately 25-30 seconds to clear 20cc of perfusate. These flow rates were consistent throughout the period of preservation.

b. Physical Appearance

The kidneys preserved with perfusate containing 20% PFOB experienced no net weight gain. The kidneys preserved in the same perfusate but without the PFOB experienced a slight discoloration over time and had net weight gain of approximately 20%.

c. Histology

Blinded histological studies revealed that all the kidneys were well preserved. There was no detectable difference between the kidneys preserved in basal solution with PFOB supplementation and those kidneys maintained without the PFOB.

d. Oxygen Utilization

The average oxygen unloading was calculated as the difference between the PO_2 from the arterial line feeding the kidney and PO_2 from the venous overflow.

	without PFOB	20% PFOB
average O_2 unloading mmHg	42 mmHg	62

Conclusion

PFOB supplementation does not appear to be required in the case of static storage. This histologic finding is not surprising since any oxygen being provided by the PFOB would be quickly depleted and no provision was made for

reoxygenating the perfusate. The basal perfusate is equivalent to the PFOB supplemented perfusate for preservation using simple static storage at 25°C.

When using pulsatile preservation at 32°C, supplementation with PFOB is beneficial. Of particular importance is the need to flush with the PFOB supplementation. The anoxia during flushing may have initiated anaerobic respiration and the subsequent observed difference in the ability of the kidneys without PFOB to utilize oxygen during the first hour of preservation. The flow dynamics were similar in both sets of kidneys; therefore, the PFOB supplementation did not affect the renal resistance over the period of preservation. The average PO_2 of the basal perfusate was 132mmHg and with PFOB supplementation this was increased to 156mmHg. The kidneys preserved in perfusate supplemented with PFOB utilized 20mmHg more O_2 than kidneys preserved without PFOB supplementation. This increase in O_2 utilization is significant, since the increase raised the O_2 unloaded to a more physiologic equivalent. While PFOB supplementation is not necessary to preserve the tissue histologically, it does appear to be beneficial in supporting optimum respiration of the kidneys during preservation.

EXAMPLE 2

Renal Preservation Without Extreme Hypothermia

To determine whether the liquid fluorocarbon supplemented preservation solution could be beneficial in maintaining flow characteristics and histology in kidneys after circulatory arrest, the following study was performed. 20 canine kidneys were flushed *in situ* within 60 minutes of cardiac arrest. The organs remained *in situ* for 1 to 8 hours without recirculation or hypothermia. Controls consisted of 5 kidneys flushed *in situ* with ViaSpan™ at 4°C, which represents state-of-the-art preservation, and 5 kidneys receiving no treatment. The remaining 10 experimental canine kidneys were flushed *in situ* with the perfusate of the present invention. The kidneys were then removed, reflushed with either the control or new

perfusate solution, and pumped on a Waters MOX-100 unit at the appropriate temperature: 4°C for ViaSpan™, and 25-32°C for the perfusate of the present invention.

Results

In contrast to the control kidneys which could not be pumped, the kidneys flushed *in situ* with the perfusate of the present invention could be reflushed and pumped. Even after 45 minutes of warm ischemia, followed by an *in situ* time of 8 hours after flushing with the perfusate of the present invention, pumping revealed acceptable *in vitro* flow dynamics. The histological findings showed no evidence of tubular or glomerular damage in the kidneys preserved in the perfusate of the present invention. In contrast, the tubules of the control kidneys showed severe interstitial edema and tubular karyolysis, along with swollen hypercellular glomeruli.

These results indicate that the perfusate of the present invention can support organ viability during preservation using moderate hypothermia. Thus, the perfusate solution and method for its use may help expand the organ donor pool to include nonheartbeating cadavers.

EXAMPLE 3

Oxygen Utilization During Preservation

To determine the oxygen utilization of the kidneys during preservation, the following study was performed. Canine kidneys were harvested using a midline incision. The kidneys were isolated and the renal arteries were cannulated. The kidneys were flushed with approximately 250cc of the basal flush solution supplemented with 20% PFOB v/v (as a 90% emulsion). After flushing, the kidneys were placed on the MOX-100 preservation system and preserved at 32°C at a systolic pressure of approximately 65mmHg. Blood gas specimens were collected at regular intervals in glass syringes. Samplings at each time point included the arterial feed line along the venous outflow. All testing was performed in duplicate. The PO_2 values were determined using an ABL 300 radiometer by Copenhagen.

Results

	Perfusate without PFOB	Perfusate with PFOB
Flow	92 cc/min	48 cc/min
Temperature	32°C	32°C
PFOB concentrate	0%	20% vol of 90%emulsion
PO_2 Arterial	132mmHg	156mmHg
Venous	90mmHg	94mmHg
Difference	42mmHg	62mmHg
O_2 consumption	0.12ml/min	0.266ml/min

FIGURE 1 shows the PO_2 values in kidneys preserved in basal solution alone. FIGURE 2 shows the PO_2 values in kidneys preserved in basal solution supplemented with 20% PFOB.

Conclusion

The PO_2 was higher in those kidneys preserved using a preservation solution supplemented with a liquid fluorocarbon emulsion. Average oxygen deposition in the kidney was 62mmHg and ranged from a low of approximately 40mmHg to a high of 72mmHg. The unloading of the O_2 during preservation was found to be consistently within the physiologic range for respiring tissue.

EXAMPLE 4

How Long Postmortem is the Kidney Salvageable?

In order to determine the time frame postmortem in which kidneys can be salvaged, four questions needed to be addressed: (1) without intervention, how long postmortem would the vasculature remain open *in vivo*; (2) how would flushing with ViaSpan™, which represents state-of-the-art preservation, affect the salvageability of the kidneys; (3) how would the preservation technology of the present invention compare to ViaSpan™; and (4) since complete flushing of an

organ *in vivo*, prior to harvest, would be difficult to achieve, how effective would partial flushing be in keeping the vasculature open and preserving the kidney until harvest. For these studies, all testing was performed in duplicate and the histological evaluations were performed blindly.

a. Without intervention, how long postmortem is the vasculature unobstructed?

Without intervention postmortem, the vasculature of the kidney remains open and fair flow dynamics can be achieved at 60 minutes of warm ischemia (see Table 3). However, at two hours of warm ischemia, the vasculature is occluded and the kidneys cannot be adequately flushed. Unacceptable flow dynamics occurred, with systolic pressure of over 180mmHg and no flow. Focal dilated capillaries and Bowman's spaces were observed histologically in the kidneys with one hour of warm ischemia. The tubular damage was also apparent, including dilation, cytoplasmic vacuoles and debris along with interstitial edema. At two hours of warm ischemia, extensive glomerular damage was observed along with severe tubular damage.

Conclusion

These results suggest that for the technology of the present invention to be implemented in expanding the existing donor pool, the *in vivo* flushing of an organ must be initiated within 60 minutes of death.

b. Efficacy of *in vivo* flushing with ViaSpan™

One set of kidneys was immediately harvested postmortem, flushed with approximately 250cc of ViaSpan™ and pumped on the MOX-100 preservation system to determine a baseline for preservation with virtually no warm ischemia. A second set of kidneys were used to evaluate the ability of ViaSpan™ to preserve the organs for three hours after *in vivo* flushing. *In vivo* flushing refers to flushing that occurs while the organ remains in the body, whether or not the animal remains alive. The aorta was dissected above the left kidney and cross-clamped. The vena cava below the right kidney was likewise cross-clamped. A cannula was inserted into the aorta

and flushing with old ViaSpan™ was initiated. The outflow was collected from the vena cava. After flushing, the vessels were clamped and the organs were maintained *in vivo*, utilizing ice packs, for three hours.

Results

ViaSpan™ could not support organ preservation *in vivo*. At three hours postmortem (the minimum time required to obtain consent from next of kin), the kidneys flushed and stored *in vivo* with ViaSpan™ demonstrated glomerular and tubular damage. These kidneys reflushed poorly after harvest and exhibited unacceptable flow dynamics (see Table 3).

c. Efficacy of the basal solution flush *in vivo*

Similar to the experiments described above, the vasculature was dissected postmortem with warm ischemia times ranging from 15 to 45 minutes. The aorta and vena cava were cross-clamped and cannulated. The kidneys were flushed with approximately 200-250cc of warm basal solution supplemented with 20% PFOB v/v (90% emulsion). After flushing, the vessels were clamped and the organs remained *in vivo* for various times. After *in vivo* preservation, the kidneys were harvested, reflushed with the flushing solution and placed on the MOX-100 preservation system. The kidneys were pumped for several hours to adequately evaluate flow dynamics after the *in vivo* preservation.

Results

In contrast to the ViaSpan™ preserved kidneys, in all cases the kidneys preserved *in vivo* with the preservation solution of the present invention could be reflushed and pumped (see Table 3). The flow dynamics were equivalent at all time points with the *in vivo* preserved kidneys. The time points selected for the *in vivo* preservation ranged from one hour to eight hours postmortem; more than enough time to declare death and to obtain consent for organ donation. Of particular significance were the kidneys experiencing 30 minutes of warm ischemia postmortem prior to the initiation of *in vivo* flushing. Even after 30-45 minutes of warm ischemia, followed by three to eight hours of *in vivo* preservation,

acceptable flow dynamics were obtained. Since a major goal for preservation under these circumstances is to achieve good reflow, these results indicate we have successfully maintained the microvasculature during the ischemia subsequent to death.

d. Potential for partial flushing *in vivo*

An important consideration in the development of warm preservation technology is the fact that complete flushing of organs *in vivo*, will be difficult to achieve. Towards addressing this point, we performed experiments evaluating the affect of partial flushing on organ preservation. Similar to the experiments of 4c. described above, the kidneys were flushed *in vivo* with the basal perfusate supplemented with 20% PFOB v/v (90% emulsion). However, only 25-50cc of the flush was perfused through the renal artery. The partially flushed kidneys were stored *in vivo* for time periods ranging from three to eight hours.

Results

Partial flushing with the basal solution supplemented with 20% PFOB supported the maintenance of the vasculature for periods of time up to eight hours postmortem. Even volumes as small as 25cc was enough to keep the vasculature open and support the preservation of the glomeruli.

i) Flow Characteristics:

The kidneys reflushed acceptably well after *in vivo* storage. When placed on the MOX-100 preservation system, all kidneys exhibited a transient rise in pressure along with a reduced flow rate. Most kidneys recovered after approximately 60 minutes on the pump. At 60 minutes, the pressures usually dropped and the flow rates increased, resulting in flow dynamics similar to those obtained in normal kidneys without ischemic time.

ii) Histology:

The glomeruli were normal in all the kidneys which were partially flushed. At three hours of *in vivo* ischemia, the tubules were well preserved (see Table 3). The first signs of vacuolization of the collecting ducts was observed after five hours of *in vivo* ischemia. The first signs of focal

interstitial edema in the tubules was detected after eight hours of *in vivo* warm ischemia.

SUMMARY OF TABLE 3

PROTOCOL	HISTOLOGIC	GROSS EVALUATION	FUNCTION*
4a. Controls			
Postmortem-No RX			
1 hr.	reversible damage	normal	good
2 hr.	extensive damage	discolored, clotted	none
4b. ViaSpan™			
No ischemia (control)	normal glomeruli, dilated tubules	swelling (15%)	reversible vaso-constriction
Flushed/3 hr. *in vivo* storage	irreversible damage	swelling (30%)	none

PROTOCOL	HISTOLOGIC	GROSS EVALUATION	FUNCTION*
4c. Basal Preservation Flushed with approx. 250cc followed by:			
1hr. *in vivo*	normal	normal	good
2hr. *in vivo*	normal	normal	marginal
30 min. warm ischemia, 3 hr. *in vivo*	normal glomeruli, focal tubule dilation	normal	good
5hr. *in vivo*	normal	normal	marginal
8hr. *in vivo*	normal	normal	good
4d. Partial Flush Flushed with approx. 30cc followed by:			
3hr. *in vivo*	normal	normal	good
5hr. *in vivo*	normal	normal	marginal
8hr. *in vivo*	normal glomeruli, tubule edema	normal	good

* Flush potential/flow dynamics

TABLE 3

METHOD	HARVEST FLUSH	VASC. RESISTANCE*	HISTOLOGY
3a. Controls			
1 hr. postmortem (no RX)	250cc/10 min.	5.0	focal dilated cap., Bowman spaces & tubules vacuoles & debris, some interstitial edema
2 hr. postmortem (no RX)	occluded, no flush	N.T.	extensive glomerular swelling, tubular karyolysis & vacuoles
3b. ViaSpan™			
no ischemia	100cc/min.	O.T. = 15 60 min.=5.8 2hr.=6.0- 3hr.=6.2	glomeruli normal, focal dilated tubules with focal edema
3 hr. *in vivo* flushing	< 100cc/20min.	no flow (180mmHg)	swollen hypercellular glomeruli, tubule damage with areas of dilation & cytoplasmic vacuoles
3c. Basal Solution Flush			
1 hr. *in vivo*	250cc/3min.	O.T.=4.3 60min.=5.2 2hr.=5.3 3hr.=5.3	normal
2 hr. *in vivo*	250cc/11 min.	O.T.=1.3 60min.=0.8 2hr.=0.9 3hr.=1.11	normal
30 min. warm ischemia; 3 hr. *in vivo*	200cc/5 min.	O.T.=5.3 60min.=3.6 2hr.=2.4 3hr.=2.4	normal glomeruli, slight tubular dilation
5 hr. *in vivo*	--	O.T.=4.8 60min.=1.7 2hr.=1.7 3hr.=1.9	normal glomeruli, tubules good
8 hr. *in vivo*	--	O.T.=1.7 60min.=5.5 2hr.=4.5 3hr.=4.5	normal glomeruli, some vacuoles in the straight tubules - overall well preserved
3d. Partial Flush			
3 hr. *in vivo* (25cc)	250cc/3 min.	O.T.=1.25 60min.=4.33 2hr.=5.0 3hr.=5.0	normal glomeruli, vacuoles in the straight tubules - overall well preserved
5 hr. *in vivo* (25cc)	--	O.T.=0.5 60min.=0.4 2hr.-0.4 3hr.=0.5	normal glomeruli, tubules good, vacuolization of collecting ducts
8 hr. *in vivo* (30cc)	--	O.T.=0.9 60min.=5.1 2hr.=4.8 3hr.=4.8	normal glomeruli, some focal interstitial edema in the tubules

Conclusion

Even partial flushing with the preservation solution of the present invention is adequate to keep the vasculature open and to preserve the kidneys for time periods of up to eight hours postmortem. The small amounts of flushing solution used in these studies support the concept that even marginal *in vivo* flushing could support organ preservation *in vivo* for periods of time acceptable to declare death and acquire consent for organ donation from next of kin.

EXAMPLE 5

Additional Organ Preservation Studies

To further determine the efficacy of the preservation solution of the present invention in preserving organs using warm organ preservation technology, three canine renal autotransplantations were performed. The first evaluated the role of the liquid fluorocarbon emulsion alone. The second evaluated the role of the basal solution alone. The third served as the control, consisting of a solution containing the basal solution supplemented with the liquid fluorocarbon emulsion.

In each dog, the left kidney was nephrectomized for the preservation studies. The intrinsic kidney maintained the dog during the period of *in vitro* preservation. Just prior to reimplantation, the intrinsic kidney was nephrectomized. The preserved kidney was reimplanted in the capsule of the intrinsic kidney which had just been removed. At no time were hypothermic conditions employed, not even during the vascular reconstruction.

Results

a. Perfluorocarbon in saline - The kidney was flushed with a solution consisting of 20% v/v of 90% PFOB emulsion in a saline solution. The renal artery was cannulated and the kidney was placed on a MOX-100 unit for organ preservation at 32°C for five hours. The kidney developed a patchy

appearance during the flushing, but flushed well. The kidney demonstrated poor flow dynamics within a few minutes of being placed on the preservation system. Visible swelling developed within the first thirty minutes of preservation, along with discoloration of the lobes. The flow dynamics continued to deteriorate during the preservation period, with periods of pronounced elevations in pressures. By two hours, the kidneys demonstrated pronounced swelling with pressures in excess of 140mmHg. At four hours, the kidney was severely discolored, demonstrated virtually no vascular flow, and had begun to leak around the cannula. At five hours of preservation, the perfusion was discontinued. The kidney was discolored, endemic, more then twice its original size, and demonstrated no flow. The unacceptable flow dynamics lead to a decision not to transplant.

The kidney was evaluated histologically. The glomerular capillaries and the blood vessels were found to be dilated. While the tubules were not necrosed, substantial interstitial edema was found.

b. Basal Perfusate Alone - The kidney was flushed with a solution consisting of basal solution alone. The kidney flushed well, 500cc in less than eight minutes. The renal artery was cannulated and the kidney was placed on a MOX-100 unit for organ preservation at 32°C for five hours. The kidney perfused well throughout the period of preservation without alterations in pressure or flow. At three hours the kidney developed a splotchy appearance with areas of whitish discoloration, which may have been caused by leaky capillaries. After five hours of preservation, the kidney was autotransplanted. Anastomosis time was prolonged, more than 45 minutes. The kidney reperfused well, exhibiting good turgor. The kidney produced urine almost immediately after the circulation was restored. The dog voided 30 minutes after transplantation and again at 3 hours post-transplantation. The dog developed bloody urine the next

morning and had a serum creatinine level of 4.7mg/dl.

The kidney demonstrated a post-mortem autolysis and a band of interstitial infiltration of polymorphonuclear cells. These results were suggestive of reperfusion injury, secondary to the prolonged period of anoxia during the vascular reconstruction.

c. Basal Perfusate Supplemented with Perfluorocarbon - The kidney flushed well, in less than 8 minutes. The renal artery was cannulated and the kidney was placed on the MOX-100 unit with less than 15 minutes of warm ischemia. Some initial vasoconstriction was detected during the first hour of preservation, along with diminished flow rates. The pressure dropped at 3 hours and the flow dynamics remained constant for the remaining period of preservation. After five hours of preservation, the kidney was autotransplanted. The kidney pinked up immediately upon reperfusion, much faster than with the basal solution alone. The turgor was excellent and urine was produced immediately. The dog continued to produce large quantities of urine during the postoperative period. The serum creatinine following transplantation was 4.1mg/dl.

Some vacuolization of the cytoplasm of the proximal tubules was detected, otherwise the kidney appeared normal upon histological examination.

Conclusion

Preservation of the canine kidney using the basal perfusate supplemented with PFOB was superior to both preservation using PFOB alone and preservation using the basal solution alone. The basal perfusate alone supports good preservation in kidneys *in vitro* in terms of viability, histology and flow dynamics. However, upon autotransplantation there is substantial infiltration of polymorphonuclear cells. This is indicative of endothelial damage which is probably due to reperfusion injury. The reperfusion injury may be secondary to the anoxia during the

reanastomosis which can range from 20 to 45 minutes. Preservation of the kidney using the perfluorocarbon supplemented basal solution totally eliminates the reperfusion injury, provides a higher oxygen tension, and supports better preservation as demonstrated by better reperfusion upon autotransplantation, increased urine production, and better postoperative chemistries.

CONCLUSION

The results from these studies demonstrate the significance of the technology of the present invention. The histological studies indicate that with current technology, using ViaSpan™ and optimized cold preservation techniques, dilation of the tubules was detected along with edema. Therefore, even optimized cold preservation leads to tubule damage. In contrast, the warm preservation model using the preservation solution of present invention supported normal glomerular and tubule histology, with no dilation or edema at the same time period of preservation. These findings alone support the significance of the warm preservation technology.

More importantly, ViaSpan™ could not be utilized for *in vivo* preservation postmortem. *In vivo* flushing with ViaSpan™, followed by just three hours of *in vivo* cold preservation, lead to swollen glomeruli (irreversible damage) and severely damaged tubules. In contrast, the results with the warm preservation technology are startling. After eight hours of *in vivo* preservation, postmortem, the kidney histology demonstrated normal glomeruli, and slight vacuolization in the straight tubules. Overall these kidneys were well preserved. Eight hours of *in vivo* preservation is ample time to declare death and to obtain consent from next of kin for the organ donation.

Since complete *in vivo* flushing may be difficult to achieve, the efficacy of partial flushing is very significant. The results indicate that with even marginal flushing, eight hours of *in vivo* preservation can be achieved

postmortem with only marginal tubule damage (reversible). In fact, the tubule damage observed following partial flushing and eight hours of subsequent *in vivo* preservation was equivalent to the tubule damage observed with the ViaSpan™ controls, employing no *in vivo* preservation at all.

These studies also demonstrate that warm preservation technology supports oxygen utilization at a near physiologic range in the preserved kidneys. Approximately 60mmHg of oxygen is unloaded during *in vitro* preservation using the basal perfusate supplemented with 20% PFOB v/v (as a 90% emulsion). While the basal perfusate in the absence of PFOB supplementation still provided a high PO_2, the oxygen utilization was approximately 20mmHg lower than with PFOB supplementation. Therefore, PFOB supplementation is important in supporting optimized respiration. Initial flushing with PFOB supplementation is very important. The kidneys flushed without PFOB supplementation were found to consistently utilize less O_2, even in the presence of a high oxygen tension. The initial *in vivo* flush with PFOB supplementation is important to avoid the anoxia early on and the subsequent institution of glycolysis.

It should be understood that the embodiments and examples of the present invention, as described herein, are for purposes of illustration only, and not limitation.

WHAT IS CLAIMED IS:

1. A method for preserving an organ prior to transplantation, comprising the steps of:

flushing an organ with a preservation medium comprising an aqueous solution having an osmolarity of from about 300 to about 1000 mOsM and emulsified liquid fluorocarbon, wherein said fluorocarbon comprises between about 1% and 50% v/v of said preservation medium; and thereafter

storing said organ at a temperature between about 18°C and about 35°C for at least one hour.

2. The method of Claim 1, wherein said flushing step is performed *in vivo*.

3. The method of Claim 1, wherein said flushing step and said storing step are performed while said organ remains *in situ*.

4. The method of Claim 1, wherein said flushing step and said storing step are performed while said organ remains *in vitro*.

5. The method of any of Claims 1-4, further comprising the step during said storing step of continuously perfusing said organ with said preservation medium.

6. The method of any of Claims 1-5, wherein said aqueous solution comprises buffered basal medium, impermeant, mucopolysaccharide, and magnesium.

7. The method of any of Claims 1-6, wherein said aqueous solution further comprises buffered basal medium supplemented with components, or functional equivalents thereof, selected from the group consisting of amino acids, ions, physiological salts, colloids, serum proteins, carbohydrates, lipid, attachment factors, and growth factors.

8. The method of any of Claims 1-7, wherein said aqueous solution comprises a solution having an osmolarity of from about 400 to about 900 mOsM.

9. The method of any of Claims 1-8, wherein said

aqueous solution comprises a solution having an osmolarity of from about 350 to about 380 mOsM.

10. The method of any of Claims 1-9, wherein said fluorocarbon comprises between about 5% to about 40% v/v of said preservation medium.

11. A hyperosmolar preservation solution which supports the preservation of organs without hypothermia, comprising:

an aqueous solution having an osmolarity of from about 300 to about 1000 mOsM and emulsified liquid fluorocarbon, wherein said fluorocarbon comprises between about 1% and 50% v/v of said preservation solution.

12. The hyperosmolar preservation solution of Claim 11, wherein said aqueous solution comprises buffered basal medium, impermeant, mucopolysaccharide, and magnesium.

13. The hyperosmolar preservation solution of any of Claims 11-12, wherein said aqueous solution further comprises buffered basal medium supplemented with components, or functional equivalents thereof, selected from the group consisting of amino acids, ions, physiological salts, colloids, serum proteins, carbohydrates, lipid, attachment factors, and growth factors.

14. The hyperosmolar preservation solution of any of Claims 11-13, wherein said aqueous solution comprises a solution having an osmolarity of from about 400 to about 900 mOsM.

15. The hyperosmolar preservation solution of any of Claims 11-14, wherein said aqueous solution comprises a solution having an osmolarity of from about 350 to about 380 mOsM.

16. The hyperosmolar preservation solution of any of Claims 11-15, wherein said fluorocarbon comprises between about 5% to about 40% v/v of said preservation medium.

17. The hyperosmolar preservation solution of any of Claims 11-16, wherein when the solution is used for initial

flushing of an organ, said solution further comprises mannitol.

18. The hyperosmolar preservation solution of Claim 17, wherein said mannitol is present at a concentration of from about 1 to about 100g/L.

19. The hyperosmolar preservation solution of any of Claim 11-18, wherein the impermeant is cyclodextrin.

20. The hyperosmolar preservation solution of Claim 20, wherein said cyclodextrin is present at a concentration of from about 0.1 to about 1g/L.

21. The hyperosmolar preservation solution of any of Claims 11-20, wherein the mucopolysaccharide is chondroitin sulfate.

22. The hyperosmolar preservation solution of Claim 21, wherein said chondroitin sulfate is present at a concentration of from about 1 to about 50mg/L.

23. The hyperosmolar preservation solution of any of Claims 11-24, wherein said magnesium is in the form of magnesium sulfate.

24. The hyperosmolar preservation solution of Claim 23, wherein said magnesium sulfate is present at a concentration of from about 0.08 to about 4g/L.

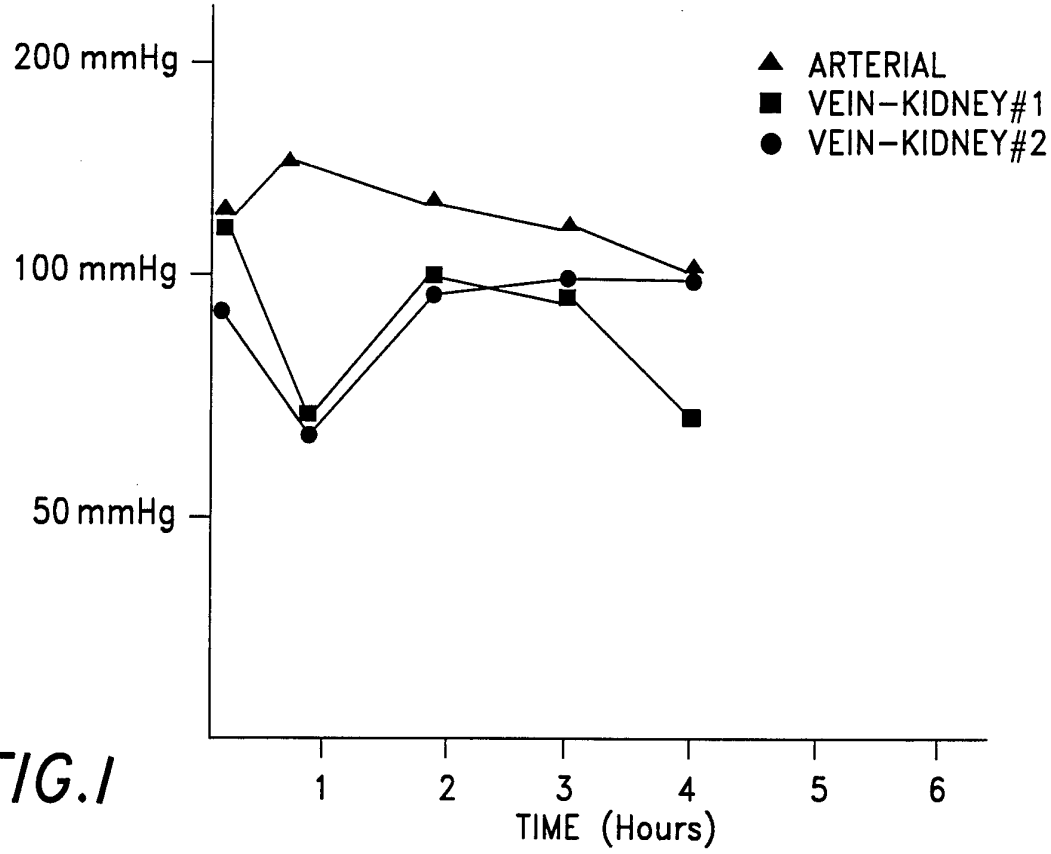
200 mmHg
100 mmHg
50 mmHg
ARTERIAL
VEIN–KIDNEY#1
VEIN–KIDNEY#2
1
2
3
4
5
6
TIME (Hours)

FIG.1

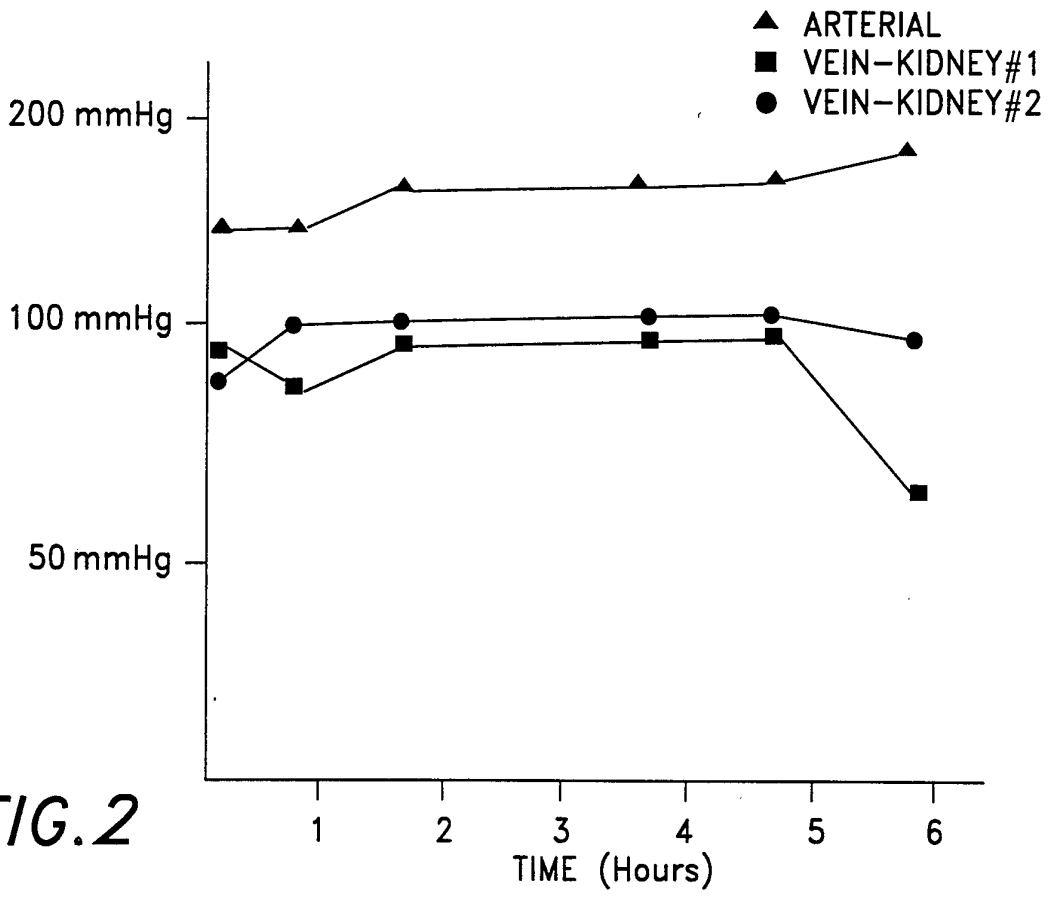
200 mmHg
100 mmHg
50 mmHg
ARTERIAL
VEIN–KIDNEY#1
VEIN–KIDNEY#2
1
2
3
4
5
6
TIME (Hours)

FIG.2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/02831

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A01N1/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 114 932 (T.M. RUNGE) 19 May 1992 see claims ---	11, 13-16, 19,20, 23,24
X	EP,A,0 033 402 (THE GREEN CROSS CORPORATION) 12 August 1981 see claims see page 7, line 20 - line 21 see page 8; table 1	11,13, 16,23,24
A	see example 5 --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
4 July 1994	20. 07. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Decorte, D

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 106, no. 25, 22 June 1987, Columbus, Ohio, US; abstract no. 210441c, CHEN H. ET AL. 'fluorocarbon preservation of kidney transplants' see abstract	11
A	& CHIN. MED. J., vol.99, no.11, 1986, BEIJING pages 871 - 878	1-10
A	US,A,4 920 044 (P.D. BRETAN) 24 April 1990 see claims 1,2	1-24
A	SURGICAL CLINICS OF NORTH AMERICA, vol.66, no.3, 1986, PHILADELPHIA pages 617 - 632 D.E. PEGG 'organ preservation' see page 627, line 9 - line 13	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US 94/02831

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-5114932	19-05-92	NONE	
EP-A-0033402	12-08-81	AT-T- 4572	15-09-83
US-A-4920044	24-04-90	NONE	