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# Polyethylene glycols and organ protection against I/R injury

## *Polyéthylène glycol et protection des lésions d'ischémie-reperfusion*

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**Summary**
During the organ transplantation process, conservation solutions must address responses to the physiologic organ preservation and prevent ischemia-reperfusion injuries. The use of colloids seems beneficial especially for long ischemia time compared to the impermeant molecules used for short time. The colloids family includes molecules as hydroxyethyl starch (HES), albumin, dextran or polyethylene glycol (PEG).
In this review, the authors describe the rational for PEG use, its potential immunomodulatory effect and the main results of its experimental and clinical use.


**MOTS CLÉS**
Solution
de preservation ;
Polyethylène glycol ;
Ischémie-reperfusion ;
Immunomodulation

**Résumé**
Pendant le processus de transplantation d'organes, les solutions de conservation sont utilisées pour répondre aux modifications physiologiques dues à la conservation et prévenir des lésions d'ischémie-reperfusion. L'utilisation de colloïdes semble bénéfique, surtout en cas de période d'ischémie froide longue par rapport aux molécules utilisées pour des durées d'ischémie froide courtes. La famille des colloïdes comprend des molécules comme l'Hydroxyéthyle Amidon (HES), l'albumine, le dextran ou le polyéthylène-glycol (PEG).
Dans cette revue, les auteurs décrivent le rationnel scientifique pour l'utilisation du PEG dans les solutions de conservation, son effet immunomodulateur potentiel ainsi que les principaux résultats de son utilisation expérimentale et clinique.


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## Introduction

During the organ transplantation process, conservation solutions must address responses to the physiologic organ preservation and prevent ischemia-reperfusion injuries [1-3]. The organ conservation sequence during the ischemic period contributes to the induction of more or less reversible injuries such as decrease of ATP (Adenosine TriPhosphate) production, acidosis due to anaerobic glycolysis, cellular edema, and mitochondria and cellular integrity alterations [4-7]. The role of the preservation solution is to reduce the occurrence of these events in order to decrease the importance of cell damage and inflammation, to preserve the functionality and integrity of the graft [1-3].

## Necessity of colloid in graft conservation solution

During the cold ischemia time, corner stone of the organ conservation, major injuries affect the organ metabolism, one the most important of which is tissue edema [8]. In the extracellular conservation solution, the presence of colloids exerting an oncotic pressure to prevent edema is therefore essential to optimize the quality of graft integrity preservation [8]. It is well established that the use of a colloid free solution, such as Eurocollins, in kidney graft preservation induces serious edema and irreversible organ integrity disturbances [9]. Beyond cell death, one of the most dangerous side effects of cell swelling is the «no-reflow» phenomenon. Indeed, at reperfusion, the cellular edema induces vascular bed compression, decreasing graft reperfusion, and consequently promoting the «no-reflow» phenomenon [10].

The colloids family includes molecules as hydroxyethyl starch (HES), albumin, dextran or polyethylene glycol (PEG). Colloids cannot cross the cell membrane and prevent, by oncotic pressure, cellular swelling. The use of colloids seems beneficial especially for long ischemia time compared to the impermeant molecules used for short time. The HES contained in the standard University of Wisconsin solution has tubular toxicity [11] and induces aggregation of red blood cells [12]. These HES inconvenients led researchers to reconsider the choice of colloid for organ conservation.

## PEG as colloid in organ conservation

Polyethylene glycol (non-activated form) is a linear polymer of ethylene oxide with a hydroxyl terminal. The longer chains are also referred as polyethylene oxide:

$HO\text{-}(CH_2CH_2O)_nCH_2\text{-}CH_2\text{-}OH$.

The molecular weight of this molecule depends on the number of ethylene oxide group.

PEG is a synthetic, non-toxic and non-immunogenic molecule, currently used in many applications especially in the food, biology and pharmaceutical industries.

PEG is soluble in water and in aqueous medium, and the polymer chains are highly hydrated, each monomer binding 2-3 water molecules.

At the end of the seventies, PEG was described as a molecule limiting cell swelling during storage of tissue sections of rat heart [13]. The presence of PEG 8 kDa also suppresses cell swelling of rat isolated hepatocytes and reduces cell death induced by 24 hours of hypothermic preservation [14]. Furthermore, in another model of rat hepatocytes conservation, maintenance of cell viability induced by the presence of the PEG colloid is associated with a preservation of actin microfilaments cortical organization and protection of the microtubule structure [15].

The first use of PEG for whole organ conservation was published in 1989. For this work, Wicomb and Collins group replaced, in University of Wisconsin solution, the colloid HES by the colloid PEG 20kDa (50 g/L), to use in a rabbit heart preservation model. Results showed that the cardiac function was better preserved with the use of PEG [16]. Then in 1991, this same group proceeded to the first use of polyethylene glycol during the transplantation process. In this pioneer clinical study of 20 patients, they observed a decrease in the number of rejection episodes after transplantation of hearts preserved ex vivo with a solution containing PEG 20kDa. The heart graft survival at 1 year was prolonged to 95.5% with the PEG solution compared to 50% with Modified St Thomas without PEG [17]. With the first observation, Collins et al suggested a theory of the "immunoprotective" role of PEG in the preservation of human heart before transplantation. Following this, there has been extended PEG use to experimental preservation of the liver [18], intestine [19] and pancreas [20]; where similar immunoprotective results were observed.

Since then, several studies have focused on the development of a new organs, tissues and cells conservation solution, containing PEG, for transplantation.

## Conservation solutions containing PEG

In the middle of the nineties Eugene et al designed a normopotassic (5 mM $K^+$) preservation solution containing initially 30g/L PEG 20kDa, called "Solution de Conservation d'Organes et de Tissus" (SCOT solution) [21]. SCOT was firstly used for transport and cryopreservation of human large arteries. The results showed allograft resistance to infection and mechanical stability close to fresh allograft arteries. Then, beneficial effects of this solution were obtained on renal function and structure during 48-hour of cold storage preservation [22]. Furthermore, benefits of this solution containing PEG 20kDa have been observed in different experimental models of organ conservation. An experimental work showed better vascular resistance and a decrease in cellular and mitochondrial swelling with the PEG solution compared to UW and the Eurocollins in a pig lung ischemia-reperfusion sequence [23]. On the rat liver and kidney extracorporeal conservation, studies have also shown the benefits of the PEG solution on preserving the integrity of the organ [24, 25]. Several kidney conservation studies have shown better graft preservation and decreased ischemia-reperfusion injuries such as immune reaction and fibrosis using this PEG solution in a porcine kidney transplantation model [9, 22, 25-29]. In this same porcine renal auto-transplantation model, the use of PEG 20kDa during organ extracorporeal preservation permitted an important reduction of MHC class I and II expression in epithelial tubule cells, a diminution of VCAM1 expression and

a limited infiltration of macrophages/monocytes and $CD4^+$ T lymphocytes, 15 days after transplantation [9, 26, 27, 29]. Also, the conservation benefits of PEG showed protective long term effects, such as the decrease of fibrosis and TGFβ signaling in an pig kidney allotransplantation model [28, 30]. In a murine Langerhans islet allotansplantation, the use of PEG 20kDa and 35kDa on tissue isolation and conservation is correlated with better islet yield, an improvement of graft function recovery and allograft survival prolongation without immunosupression [31, 32]. In this model, the use of PEG 20 kDa permitted significantly prolonged islet allograft survival in 2 models of allograft rejection, including a transgenic model in which >90% of the recipient CD8 TCR were specific for an antigen expressed on the graft islet beta cells [33]. This beneficial reduction of the recipient immune alloreaction is due to an important reduction of pancreatic islets immunogenicity by PEG, proved by the antigen allorecognition reduction [33].

This SCOT solution, now containing 15g/L PEG 20kDa (replacing the 30g/L original concentration), is used in human kidney and liver transplantation. The main effect of cold storage of human liver using SCOT compared to UW was the decrease of cholestasis following transplantation [34]. In clinical kidney transplantation, the SCOT solution was used for in situ flush and/or static preservation, and data related a decrease of delayed graft-function (not significant) [35]. In human pancreas conservation, the use of a colloid free solution such as the Celsior solution induced cell swelling and pancreas edema after only four hours of cold storage, these abnormalities were delayed when the donor pancreas was perfused with SCOT Solution [36].

Another conservation solution containing 1g/L of PEG 35kDa, called Institut Georges Lopez-1 (IGL-1), showed protective effect in organ or tissue transplantation. The 1g/L PEG 35 kDa in HES free UW solution improved, at 4 °C, the organ flush by a reduction of red blood cells aggregation compared to UW containing 50g/L HES [37]. The use of IGL-1 during 24h of kidney cold storage had beneficial effect such as decreased cellular apoptosis and reduction of MHC class II expression after porcine kidney autotransplantation [38].

IGL-1 is now currently used in clinical transplantation. In 2011, results from an European cohort of liver transplantation, showed that partial liver grafts preserved with IGL-1 (n=59) had better survival than graft preserved with UW (n=1308): 90 vs 75% and 86 vs 69% at 1 and 3 years, respectively [39]. In 2009, a report from the first multi-center study of IGL-1 solution in kidney transplantation was published, and data showed lower serum creatinine levels during the 12 month follow up in the IGL-1 group. In addition, significant differences in rejection rates as well as in graft survival were observed between the UW and IGL-1 groups [40].

Recently, the POLYSOL solution has been developed for hypothermic preservation. POLYSOL is a preservation solution with low viscosity and a high buffering capacity, which contains 60 components, consisting of impermeants, antioxidants, vitamins, energy substrates, amino acids and PEG 35 kDa at 20g/L. In a porcine model, kidneys washed-out with POLYSOL showed better preservation of structural integrity after 24 hours of cold storage compared with either UW or HTK [41], and higher values of capillary blood flow, blood flow velocity and tissue oxygen saturation values at reperfusion compared with UW solution [42]. In this pig model, histologic evaluation of warm ischemic damaged kidney grafts showed less glomerular shrinking, tubular damage, edema, inflammatory infiltration, and necrosis with POLYSOL conservation compared with HTK preserved grafts [42]. Cold storage using POLYSOL resulted in significantly better integrity and function of rat steatotic livers [43] and thus improved the preservation quality of partial liver transplantation [44].

## PEG immunocamouflage properties

The presence of PEG on the cell surface is not deleterious for cellular metabolism [9, 45, 46]. Moreover PEG preserved the cellular integrity at low temperature (4 °C) [16, 22, 47, 48]. PEG hydrates and stabilizes the cell membrane, and makes it less permeable to extracellular elements [16, 20].

The benefits of PEG to prevent cell swelling and preserve cellular integrity are now well established and demonstrated to be superior to HES. Another advantage of PEG is the reduction of host immune reaction against PEG conserved graft. This immunoprotective effect could be explained by the antigen immunomasking effect and the protein repulsive property of PEG.

Immunocamouflage relies on the modification of the cell membrane surface with non immunogenic molecules creating a barrier that prevents the antigen recognition by the recipient immune cells and antibodies [49]. The height of PEG on cell surface is due to a phenomenon of structuring water molecules over 6 to 8 layers around the PEG chain [49]. The overall dimension of this water molecules structure could explain the immunomasking role of PEG, through concealement of surface antigens. In contrast to other immunomodulatory approaches, immunocamouflage presents a more versatile effect, combining interferences with binding, allorecognition, and presentation pathways [50]. This immunomasking effect is depending on the PEG membrane attachment property.

The immunocamouflage effect of non-activated PEG depends on 1) molecular weight (chain length), 2) concentration and 3) cell surface type.

There is a critical chain length for optimal physical bonds with the cell surface, conditioning the binding space, making it more or less high on the cell surface. This PEG binding space on the cell membrane determines its protective "immunomasking" role [51, 52]. The PEG adsorption to the cell membrane is dependent on the PEG molecular weight that varies with the number of ethylene oxide group. The PEG commonly used for aggregation or fusion applications is in the molecular weight range of 8 to 10kDa [53]. However, the large chains lengths between 10kDa to 35kDa are preferentially absorbed, consequently they are more effective for the "immunomasking" antigen effect on the immunological synapse [32, 49]. The height of the immunological synapse is estimated at 15 nm. PEG 20kDa when attached on the cell membrane, has a height of about 20 nm [8, 49]. The length of the PEG chains determines their ability to hide surface antigenic molecules, in this way PEG 20kDa masks larger molecules than the PEG 8kDa. In fact, Itasaka H showed in a rat bowel transplantation model that conservation with PEG 20kDa had an immunoprotective effect related

to a significant graft survival prolongation, observation not obtained with PEG 8kDa [19].

An additional immunoprotective property of PEG, in adequation with the antigen immunocamouflage on cell surface, is that PEG molecules constitute an amphiphilic group exerting a steric repulsion, rejecting the protein binding [54]. Indeed, the presence of PEG on the surface of nanoparticles shows, in vivo, an interactive reduction with blood mononuclear cells, increasing the survival time of nanoparticles [54].

The high number of water molecules binding on high PEG chain contributes to creating a large volume or "exclusion volume", which prevents the approach of the other molecules [49].

PEG concentration, in association with the molecular weight, determines the protective effects. In static conservation, the use of PEG 20kDa (10 to 30g/L) on murine pancreatic islets preservation gives better results in term of islet allograft survival time up to 20 days compared to 35kDa (1 to 52g/L) [31]. These results are in agreement with the benefits of PEG 20kDa 30g/L in static graft conservation to significantly prolong allograft porcine kidney survival (80% at 3 months) compared to PEG 35kDa 1g/L (40% of graft survival at 3 months) [55].

PEG concentration could have some important rheologics effects depending of the vascular graft architecture. The heart vasculature accepts higher PEG concentration, more than in kidney and more than in liver. Mosbah and Zhao group showed that PEG could have some aggregation effect on *in vitro* red blood cells, and demonstrated that this effect is correlated with PEG molecular weight and concentration. As with HES, the use of PEG 20kDa > 30g/L and PEG 35kDa > 10g/L is correlated with aggregating effects on red blood cells [37, 56]. *In vivo*, these rheologics disorders could create a vascular obstruction. This notion should be integrated in the development of perfusion solution containing PEG.

PEG adsorption depends on other important parameters such as the radius of curvature and the charge of cell surface. In case of a large radius of curvature, such as on vascular wall or pancreatic islets, the PEG adsorption is more important than on a high cell surface curvature (due to a small radius of curvature) where the adsorption is limited. This explains the difference in benefits of 20kDa PEG immunoprotective effect between pancreatic islet [33] and lymphocytes cells [57]. In fact, a significant decrease of alloreaction was obtained in immune antigen detection studies with modified islet surface by PEG 20kDa [33], whereas the immunomasking effect of PEG 6kDa, 20kDa and 35kDa PEG was not observed on human lymphocytes [57]. In addition, the direct interactions of PEG with the proteins of the extracellular matrix are different on lymphocytes or islet. This could be explain the absence of immunocamouflage obtained with PEG on lymphocytes by Perrin et al [57], compared to the immunomasking effect of PEG on pancreatic islets (known for their adhesion properties) [33]. In the transplantation process, PEG must be used in the conservation to limit the graft immunogenicity, not on recipient PBMC, especially because recipient must stay immunocompetent. The model of PEG study takes an real importance, indeed PEG adsorption on *in vitro* culture of kidney proximal tubular epithelial cell do not reflect the physiological aspect of membrane tubular geometry as *in situ*. This information could explain the differences of PEG size effect observed on an *in vitro* study of our group [58].

The limitation of the non-activated PEG is that the adsorption on the cell membrane is time limited because of weak hydrogen connections. Hauet at al showed that the PEG 20kDa was measured in urine during 7 days after PEG conserved porcine kidney transplantation.

To obtain covalent attachment, the terminal hydroxyl group of a monomethoxy PEG (mPEG) can be activated with a variety of chemical reactive groups (as benzotriazolyl carbonate, succinimidyl, isocyanate,…) to form "activated-PEGs". These reactive-PEGs can bind strongly and for a long time to the cell membrane (until the cell membrane turnover).

This PEG activation improves the membrane attachement potential of low molecular weigth PEG (in difference of the non-activated PEG), and allows a better and longer time immunomasking effect. The first works by Abuchowski et al, in 1977, demonstrated the potential of mPEG to reduce the antigenicity. The mPEG 5 kDa were covalently attached to bovine liver catalase, inducing modification of 43% of the catalase amino groups. This PEGylation resulted in a reduction of antibody production and antibody reaction against the modified catalase, and enhanced survival in the blood of acatalasemic mice during repetitive intravenous injections [59].

In 1997, Scott and Murad group suggested the theory of cellular antigen camouflage by PEG [60, 61]. They demonstrated that the fixation of 3 to 8 g/L of 5kDa mPEG on mammalian red blood cells (RBC) significantly decreases RBC blood ABO group antibody binding and phagocytic destruction by heterologous phagocytes [60]. Their initial studies on xenogeneic transfusions demonstrated increased survival of mPEG-modified RBC [60]. Based on this, they speculated that similar chemical camouflage of intact cells may have significant clinical applications in both transfusion and transplantation. Following this study, Scott et al extended the same PEG benefits on blood leucocytes and splenocytes, by a demonstration of the effectiveness of immunocamouflage in preventing allorecognition on Mixed Lymphocyte Reaction (MLR) studies [62].

The presence of activated-PEG on the vascular wall reduces blood cell adhesion on endothelial cells due to the masking of proteins on the vessels surface [63-65]. Thus this attachment of PEG to the membrane cell decreases the graft immunogenicity [33, 66-69] by masking antigenic sites [51, 52, 61, 70]. These works on red blood cells had clearly demonstrated the ability of activated-PEG to mask surface antigens [51, 52, 61]. Another evidence of immunomasking properties of activated-PEG comes from the results of the Mixed Lymphocytes Reaction [62] and of mixed lymphocyte islet coculture experiment (MLIC). In 2004, In 2004, Lee et al, showed that mPEG-SPA (succinimidyl propionate) 5kDa grafted onto islet capsules, had positive effect on prevention of lymphocytes or splenocyte activation, but could not prevent the macrophages cytotoxic activation [71, 72].

The effect of activated PEG on immunocamouflage depends on 1) chain length, 2) concentration and 3) type of chemical reactive group [51].

As was detailed for non-activated-PEG, but not with the same type of attachment, there is a critical chain length and concentration for conditioning the immunocamouflage space,

and higher molecular weight are preferred [51]. Scott and Murad group demonstrated that the PEG binding space on cell membrane determines its protective "immunomasking" role [51, 52]. The use of mPEG 20kDa compared to 2kDa or 5kDa showed beneficial effects on modified red blood cells survival in mice at immunoprotective concentrations (up to 2 mM) [51].

The chemical linkers (C-, BTC-, and SPA-mPEG) used to activate the mPEG for covalent binding of the polymer to cell membrane proteins all preferentially target the lysine residue on proteins. Bradley et al showed that while C-mPEG was faster reacting than both BTC-mPEG and SPA-mPEG, they all gave comparable results after 1h [51].

However, the use of activated-PEG is only possible on free cells (PBMC) or islet, it would not be practical on a whole organ with a vascular tree. Indeed, the introduction of activated-PEG would modify the pH through caused by the chemicals reactions of active binding. This acidosis should imply to rapidly rinse the graft in order to control this deleterious pH modification, but this rinsing could be harmful to the vascular network creating rheologic disorders related to the increased perfusion pressures. In addition, covalent connections are difficult to control and create too large attachments that may induce congestion (obstruction) of the vascular network.

The mechanisms underlying the PEG-mediated immunoprotection is the global camouflaging of antigenic sites, repulsive membrane surface charge inducing attenuation of receptor – ligand binding and cell – cell interactions. The biophysical interactions of PEG on surfaces involves complex mechanisms dependent on the molecular weight, grafting concentration, target size and surface geometry complexity [73].

## Perspectives on immune tolerance

In 2007 the Lee et al work had demonstrated that activated-PEG on islets improve long-term islet allograft survival without immunosuppressive medication. In fact multiple PEGylated rat islets transplants survived for 100 days, however, non-PEGylated islets were completely destroyed within 1 week [74]. Furthermore, PEG encapsulated cells could be another alternative to immune tolerance of cell engraftment [75]. Chemical modification by coating the microcapsules with PEG improves biocompatibility by preventing fibrotic overgrowth [72].

## Conclusion

The use of high PEG >10kDa between 1g/L to 30g/L confers a major interest in organ preservation, to preserve graft integrity and to reduce graft antigen allorecognition. The PEG exclusion layer is the physical entity which gives rise to the immunocamouflage of the membrane antigens. The choice of chain length and concentration is determinant for optimal membrane stabilization and immunomasking effects.

The combination of these PEG properties provides solutions to (i) improving the preservation of the graft integrity, allowing graft function recovery, and (ii) limiting the intensity of the innate immunity, improving graft survival time benefit.

## Disclosure of interest

The authors have no conflicts of interest to declare in relation to this review.

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