Preservation methods for transplant organs

Organ quality can be improved

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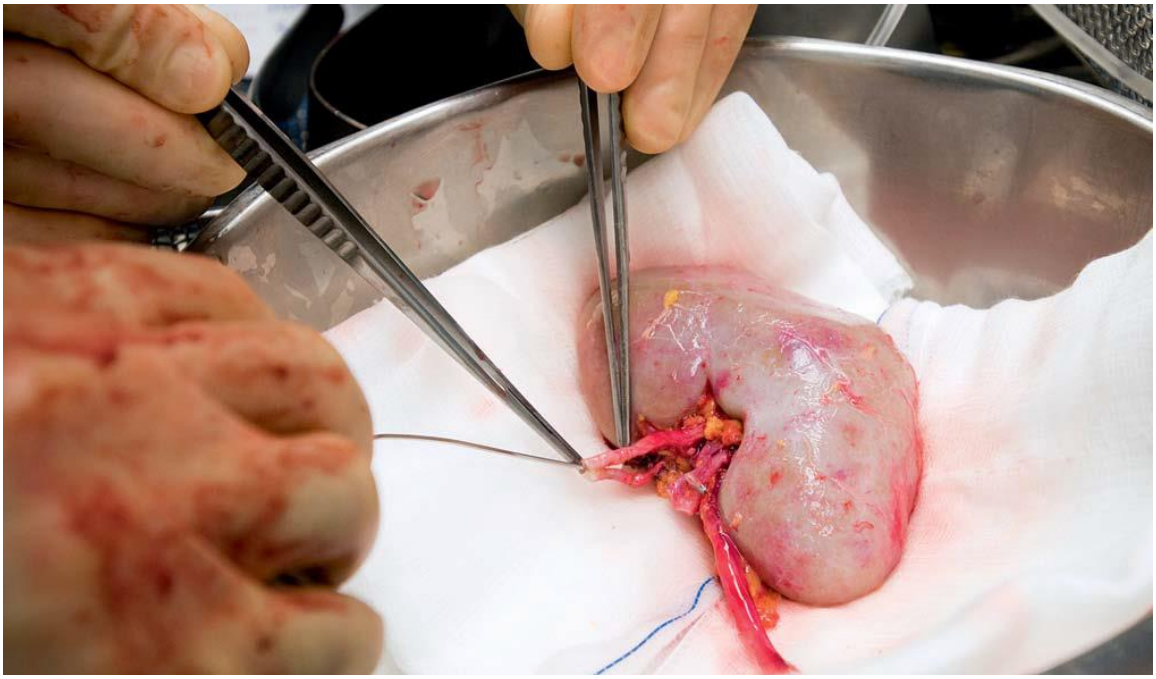
The interval between retrieval and implantation of an organ can be used not only to prevent tissue damage but also to improve graft quality. New procedures should result in a higher number of organs being viable and utilized for transplantation



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[caption: ***This explanted kidney*** *is in good condition. Standard procedure for the ischemic period is static preservation in ice-cooled solution.*]

Organ transplantation is a safe and often life-saving treatment option for patients suffering from organ failure. Advances in operating techniques, basic understanding of immunology, rejection prophylaxis/therapy and, last but not least, organ preservation have paved the way for long-term success in transplantation medicine. However, the shortage of donor organs remains a fundamental problem. Furthermore, improvements in healthcare and longer life expectancies are leading to a marked increase of older organ donors.

Major databases such as the Scientific Registry of Transplant Recipients (SRTR) in the USA illustrate these changes. Over the last 20 years, the proportion of organ donors older >50 has increased by >200% while the utilization of organs from donors <50 years of age has dropped by 76%. Therefore, utilization of marginal organs (previously defined as "Expanded Criteria Donor") is becoming increasingly common. The definition of marginal donors is predominantly based on donor age in addition to other aspects impacting organ functions.

Organs from older donors are more vulnerable and more immunogenic

Organs from older donors display very specific characteristics in terms of vulnerability and immunogenicity that can jeopardise the success of transplantation. These include age-specific compromised repair procedures and a concomitant increased risk for damages subsequent to ischemic/reperfusion injury that may occur between cessation of blood flow at the time of procurement and organ reperfusion at the time of implantation. Older organs also carry a greater risk of immunogenicity owing to numerous molecular changes such as heightened antigen presentation or increased intragraft apoptotic rates[1]. Our own research shows a higher rate of acute rejections in older and damaged transplants[2].

Much effort has been devoted in recent years to improving the quality of organs for transplantation. Those measures include organ perfusion and preservation techniques based on dynamic flow principles, mainly in the form of pulsatile perfusion. Those techniques can indeed contribute to a significant improvement in organ quality. The first clinical trials to preserve organs using machine perfusion took place more than 60 years ago[3]. However, broad clinical adoptions had been hampered by the sheer volume of equipment needed and cumbersome logistics involved. Simultaneous improvements in perfusion solutions - resulting in more effective static preservation – combined with advances in immunosuppressant drug therapy meant that the isolated effect of continuous organ perfusion could not necessarily be demonstrated when they had been introduced 60 years back. Also, more organs from younger donors had been available at those times.

In recent years, however, machine perfusion has re-gained increasing attention as a possible option to overcome the persisting shortage of donor organs and the increased availability of marginal donors (cf. eTable). To date, the most compelling evidence of the impact and benefits of machine perfusion has been provided by a prospective randomized multicentre study. In this study, both kidneys were retrieved from over 350 donors, and in each case one kidney was subjected to static cooling using preservation fluid while the contralateral kidney had been preserved by hypothermic pulsatile machine perfusion[4].

Results demonstrated a significant improvement in terms of immediate function and one-year graft survival (94% versus 90%) in kidneys preserved by pulsatile machine perfusion. Machine perfusion also yielded a significant enhanced graft survival rate after three years. A subgroup analysis of marginal organs showed an even clearer improvement in transplant survival after three years compared with a control group that underwent static preservation (86% versus 76%)[5].

Improved outcomes in the ET Senior Program

Under the Eurotransplant (ET) Senior Program, organs from donors ≥ 65 are allocated preferentially to recipients ≥ 65 with the aim to achieve improved use of older organs with short ischemic times. A study carried out under the ET Senior Program showed a marked improvement in the immediate graft function of kidneys from older donors preserved with machine perfusion, compared with kidneys that had been preserved by conventional hypothermic static preservation[6]. Within the subgroup of organs showing a delayed graft function, the advantage was particularly marked: one year after transplantation, 84% of machine-perfused kidneys were still functioning, compared with just 48% of static-preserved organs. In other words, machine perfusion yields better transplant survival rates, particularly for organs falling under the ECD criteria.

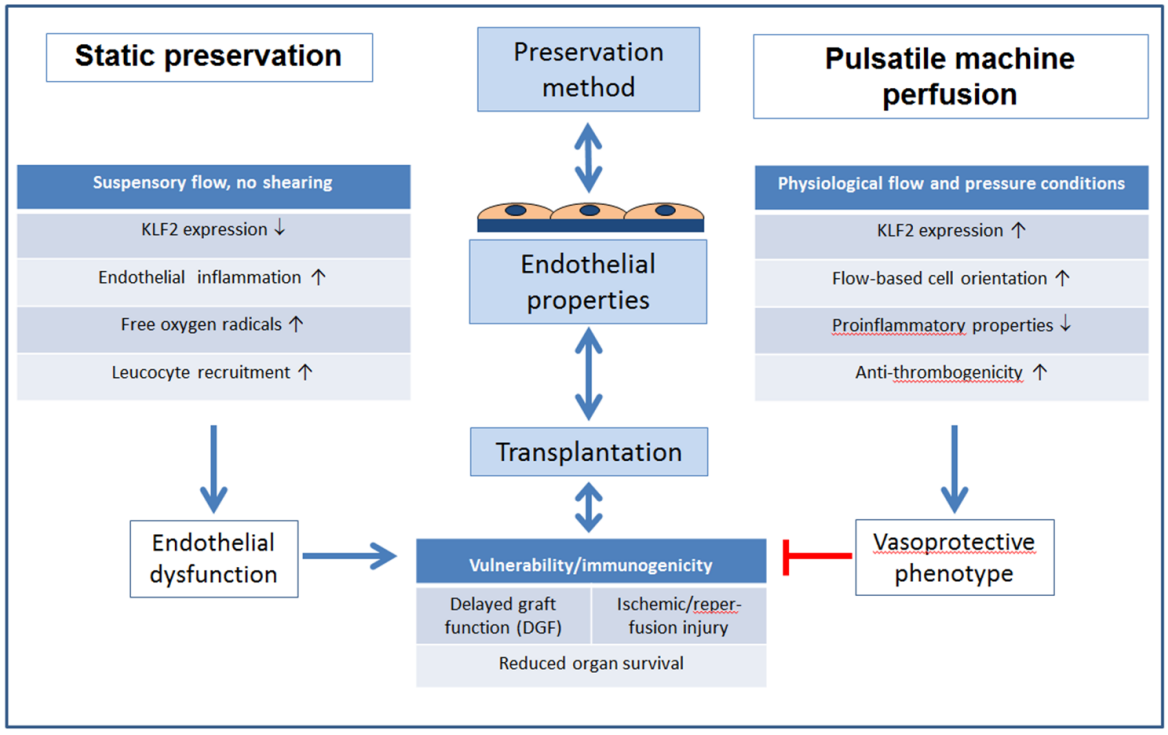
A key protective mechanism of machine perfusion seems to be the protection afforded by the endothelium. Recent own studies delineating the consequences of flow on vascular biology has demonstrated mechanistic links between pulsatile perfusion, vascular

phenotyping and gene expression patterns. A key role in maintaining vascular homeostasis is clearly played by Kruppel-like factor 2 (KLF2), an endothelial transcription factor. One possible explanation is that its function is directly dependent on pulsatile vascular flow and the resultant physiological shear stress of the endothelium. Whilst pulsatile vascular flow and shear stress show a marked endothelial expression of KLF2, cessation of pulsatile flow results in the immediate and rapid decline of KLF2 expression in the endothelium[10].

Those effects, in turn, lead to increased pro-inflammatory and thrombogenous activity of the endothelium, mechanisms which are linked to the lack of KLF2 and its endothelial protective effects on a molecular level.

Interestingly, *in vitro* studies involving human tubular epithelial cells have also been able to demonstrate the protective impact of pulsatile perfusion[11]. Those links may furthermore contribute to the clinically observed lower rates of immediate organ function with higher rates of acute rejections when transplanting older organs. (cf. diagram).

DIAGRAM: **Dynamic relationships between organ preservation, endothelial properties and transplant outcome**



Hypothermic machine perfusion thus represents a safe and, based on the data currently available beneficial alternative to static organ preservation, particularly for marginal organs.

Moreover, a number of additional new approaches aiming to optimize organ preservation are currently under way.

In the drive towards maintaining physiological conditions during the organ preservation, potential benefits of pulsatile flow under normothermic conditions have been explored. Initial studies on normothermic machine perfusion to preserve marginal kidneys have yielded promising results, with advantages in terms of immediate graft function[12]. The results of a recently published study on normothermic machine perfusion of donor hearts are likewise encouraging and prove for the first time that this preservation method may

increase the number of marginal cardiac grafts for transplantation[9]. These studies also show that innovative preservation methods can lead to the improved use of marginal organs in the extra-renal context. Organs that are not suited to transplantation when using static procedures might be viable for transplantation after preservation using dynamic procedures. In the clinical context of lung transplantation, the use of normothermic *ex vivo* perfusion led to an improved quality being reconditioned[8]. In more detail, repair processes induced by *ex vivo* perfusion have allowed damaged organs to be successfully transplanted.

WORK IN GERMANY
Studies into dynamic perfusion methods for hearts, lungs, livers and kidneys are also ongoing or planned in research institutes in Germany. Cost-benefit considerations will show whether these become routine. Germany, along with seven other countries, belongs to Eurostransplant, an organisation that allocates organs to recipients within this community. Kidneys are mostly explanted by surgeons of transplant centers in proximity of the donor hospital. This may become crucial in terms of costs and logistics since perfusion would begin immediately after explantation also if the organ would be allocated to another Eurotransplant country. For the retrieval of thoracic organs, by contrast, surgeons travel from the recipient centres to retrieve the organs themselves. Portable machine perfusion systems might have an advantage here over their static counterparts. "Dynamic perfusion procedures are a possibility once the outstanding questions have been resolved," says Dr Axel Rahmel from the Deutsche Stiftung Organtransplantation. In the United States, it is standard practice to dynamically perfuse kidneys from donors with expanded criteria. *nsi*

Ischemic damage can be compensated

Reports from Sydney describe the success of recent heart transplants after a lengthy warm ischemic period (a situation that is common in organ donation after cardiac arrest), where, after retrieval of the heart, normothermic machine perfusion was carried out. Those data have thus far only been reported during press conferences while a peer reviewed scientific report is expected very shortly.

Several other developments are also in the pipeline. The use of a novel hypothermic oxygen-enriched perfusion solution in animals has led to a reduction in ischemic reperfusion injury and reduced immunogenicity in transplanted livers by directly affecting resident immune cells, at the same time improving organ quality and attenuating rejection episodes. Thus the effects of temperature on organ preservation are scientifically and clinically interesting, but still warrant further detailed exploration.

It seems clear that organ preservation has a key role to play in efforts to overcome the shortage of organ donors while addressing changing demographics of the donor pool by making organs viable for transplantation that were previously not utilized. Hypothermic machine perfusion seems currently the best preservation option for improving organ quality. On a molecular level, there are interesting results that point to the endothelium as the main instigator for organ damage in the absence of pulsatile perfusion.

It can therefore be assumed that organ quality will continue to improve in future as new preservation methods make their appearance. Optimisation of temperature will play a key role here, as will research on the prognosis of organ functions during the *ex vivo* perfusion phase.

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Selected studies analyzing the effects of machine perfusion in various organ systems (MP = machine perfusion, SP = static preservation)

Author	Year	Organ	Patients	Study design	Results
Moes[4]	2009	Kidney	672	* prospective * multicentre * randomised	* immediate graft function ↑ (79.2% MP v 73.5% SP) * 1-year transplant survival rate ↑ (94% MP v 90% SP) * 3-year transplant survival rate↑ (91% MP v 87% SP)
Guarrera[7]	2010	Liver	40	* prospective * single centre * non-randomised	* immediate graft function ↑(95% MP v 75% SP) * Length of hospitalisation↓ (10.9 days MP v 15.3 days SP) * Transaminases ↓
Cypel[8]	2013	Lung	23	* prospective * single centre * non-randomised	* graft function ↑ (85% MP v 70% SP) * physiological functional parameters↑
Garcia Saez[9]	2914	Heart	26	* prospective * single centre * non-randomised	* maintenance of biventricular organ function in 92% of cases where marginal organs used * 96% patient survival rate after average follow-up of 8.5 months

Literature:

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4. Moers C, Smits JM, Maathuis MH, et al.: Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med 2009; 360: 7-19.

5. Moers C, Pirenne J, Paul A, Ploeg RJ: Machine perfusion or cold storage in deceased-donor kidney transplantation, N Engl J Med 2012; 366; 770-1.

6. Gallinat A, Moers C, Treckman J, et al.: Machine perfusion versus cold storage for the preservation of kidneys from donors ≥ 65 years allocated in the Eurotransplant Senior Programme, Nephrol Dial Transplant 2012; 27: 4458-63.

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9. Garcia Saez D, Zych B, Sabashnikov A, et al: Evaluation of the organ care system in heart transplantation with an adverse donor/recipient profile. Ann Thorac Surg 2014; 98: 2099-106.

10. Gracia-Sancho J, Villarreal G, Jr., Zhang Y et al.:" Flow cessation triggers endothelial dysfunction during organ cold storage conditions: strategies for pharmacologic intervention. Transplantation 2010; 90:142-9.

11. Timsit MO, Adams WJ, LagunaFernandez A, et al.: Flow is critical for maintaining a protective phenotype in renal proximal tubular cells, Am J Transplantation 2013; 13: 1617-8.

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