REVIEW

The emergence of regenerative medicine in organ transplantation: 1st European Cell Therapy and Organ Regeneration Section meeting

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SUMMARY

Regenerative medicine is emerging as a novel field in organ transplantation. In September 2019, the European Cell Therapy and Organ Regeneration Section (ECTORS) of the European Society for Organ Transplantation (ESOT) held its first meeting to discuss the state-of-the-art of regenerative medicine in organ transplantation. The present article highlights the key areas of interest and major advances in this multidisciplinary field in organ regeneration and discusses its implications for the future of organ transplantation.

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Introduction

Current treatment options for end-stage organ disease include support of organ function through dialysis, respiratory devices or ventricular pumps, lifestyle changes to slow down disease progression, and eventually transplantation. The impressive advance in knowledge of genetic editing and cellular reprogramming over the past period has led to the idea of boosting endogenous regeneration or supply *de novo* generated cells and tissues as an alternative to transplantation. However, many biological, technical and ethical challenges must be addressed before improvement of organ function through reparative therapies can be realized. To that end, the European Cell Therapy and Organ Regeneration Section (ECTORS) was established in 2018 within the European Society for Organ Transplantation (ESOT). This section finds its origin in the former Mesenchymal stromal cell in Solid Organ Transplantation (MiSOT) study group [1-3], but also includes experts on organ machine perfusion, pluripotent stem cells including human embryonic stem cells and induced pluripotent stem cells (iPSC), and organoids. The aim of ECTORS is to advance the knowledge of organ regeneration through bringing together physicians and basic scientists from the regenerative medicine and transplantation fields.

In this article, we discuss the state of the art and recent advances in organ regenerative medicine research. This includes repair of transplant organs before and after transplantation, and of diseased organs in patients with end-stage organ failure.

Organ regeneration

Organ regeneration ultimately involves the reinstitution of multiple types of cells and supportive matrix with the aim to restore integrity and function of diseased organs. If successful, organ regeneration could eventually make organ transplantation obsolete. Organ regeneration can be approached from different angles, depending on the status of the injured organ and the tools available. One approach is to make use of the intrinsic regenerative potential of organs and activate endogenous progenitor cells through pharmacological or cellular intervention or through manipulation of organ physiology. Experiments in this direction are ongoing and promising tools are under development, including machine perfusion and mesenchymal stromal cell (MSC) therapy that may boost organ regeneration. Nevertheless, many hurdles have to be overcome, including ethical, financial, logistic and mechanistic challenges, before these tools can be applied for effective organ regeneration. Another approach is to build organs or part of organs from scratch. While it is highly unlikely such techniques will be feasible in the short-term, this concept brings with it an immense potential to regenerate organs of all ages and in all developmental and disease states.

Organ machine perfusion

In recent years, progress has been made with preservation of transplant organs on hypothermic machine perfusion [4,5]. Indeed, in the Netherlands and in the UK, hypothermic machine preservation has become standard practice for all kidneys from deceased donors. The benefit of oxygenation of kidneys on hypothermic machine perfusion for kidney function has been demonstrated in seminal studies [6,7]. A multicentre randomized trial in heart transplant patients demonstrated that patient and heart survival after warm perfusion of heart transplants was noninferior to cold storage and offers possibilities to assess the metabolic status of heart transplants [8]. To develop machine perfusion into a technology that does not only preserve but also regenerates organs, further adaptations are required, including normothermia. Normothermic machine perfusion (NMP) is designed to preserve organs under physiological conditions allowing the restoration of cellular metabolism and replenishment of ATP [9-11]. It also has the potential as a platform for assessment of the donor organ before

transplantation and for pretransplant therapeutic interventions to enhance organ quality [12]. The molecular mechanisms underlying NMP involve oxidative phosphorylation, which is amongst the pathways most significantly up regulated during NMP. NMP furthermore leads to increased expression of erythropoietin and haemeoxygenase-1 and, in addition, a number of immunological pathways including TNFα signalling via NFκB are also significantly up regulated. The inclusion of a cytosorb column into the perfusion circuit to adsorb cytokines and chemokines may be used to reduce the level of pro-inflammatory cytokines [13]. Other inflammatory pathways up regulated during NMP, such as the JAK/STAT pathway, are associated with ischaemic conditioning [14]. It may be through these processes that NMP protects against ischaemia reperfusion injury in kidney transplantation.

Also within the field of liver transplantation, the implementation of machine perfusion is advancing. Conventional static cold storage is insufficient for the preservation of high-risk livers and does not allow for the evaluation of residual functionality of the liver graft. By applying heart-lung machine technology to the isolated organ, liver machine perfusion allows for better preservation and functional evaluation of the liver [15]. Liver machine perfusion can either replace conventional storage [16] or can resuscitate grafts previously preserved in a conventional manner [17]. Additionally, by repeatedly sampling the perfusate and bile produced during machine perfusion and measuring simple serum biomarkers, it is possible to assess the functionality of a graft and to predict the risk of post-transplant complications [18].

Regeneration cannot be achieved during a few hours of machine perfusion, but regenerative processes can be initiated *ex vivo* while organs are on machine perfusion. Preservation of organs through machine perfusion provides an excellent opportunity to apply regenerative therapies directly to the organs, including cellular and molecular interventions. The application of microvesicles or exosomes from regenerative cells such as MSC is an appealing setting to be further investigated [19]. Furthermore, the modulation of the expression of microRNAs with the aim to induce repair may also represent a yet unexplored approach to donor organ treatment outside the donor.

Mesenchymal stromal cell therapies

Mesenchymal stromal cell has immunomodulatory and tissue regenerative properties and therefore make them an attractive therapeutic candidate within organ transplantation. Over the last decade, a number of studies have investigated the intravenous administration of autologous MSC after human kidney transplantation and demonstrated safety, feasibility and an indication for immunosuppressive capacities of autologous bone marrow derived MSC [20,21]. It appears that the timing of MSC treatment and the concurrent immunosuppressive medication influences the effects of MSC [22,23]. It has for instance been shown that pretransplant but not post-transplant administration of MSC is effective in prolonging allograft survival in murine models and there is data suggesting that immunosuppressive drug such as tacrolimus, mycophenolic acid and rapamycin differentially affect MSC proliferation and immunomodulatory capacity [22,24,25]. With the first exploratory studies completed over 7 years ago, long-term safety profiles of MSC treatment are available. To date, there is currently no evidence for adverse effects in kidney transplant patients long after MSC administration [26]. In addition, autologous MSC therapy promoted a sustained and long-lasting pro-tolerogenic immune environment, particularly remarkable in one kidney transplant patient. This patient was successfully weaned off immunosuppressive drugs and is now almost two 2 years free from rejection with optimal kidney allograft function [27]. Treatment with autologous bone marrow MSC in kidney recipients is now moving from Phase I to Phase II trials. A currently ongoing phase 2 study is testing the hypothesis that MSC in combination with everolimus immunosuppression facilitates withdrawal of the calcineurin inhibitor tacrolimus, reduce fibrosis and decrease the incidence of opportunistic infections compared to standard tacrolimus medication [28]. The primary end-point of this study is fibrosis measured by quantitative Sirius Red scoring at 6 months after transplantation. Results will provide information on whether MSC in combination with everolimus allows graft survival with preservation of renal structure and function.

Allogeneic MSCs offer an alternative cellular therapeutic strategy to autologous therapies. Allogeneic MSC can be expanded on a large scale and are amenable to cryopreservation. A number of commercial organizations are actively developing allogeneic MSC-based products as 'off-the-shelf' cellular therapies. Such a product would be available as required and be used by those treatment centres that lack a dedicated GMP cellular-production facility. However, there is evidence that allogeneic MSC can elicit an anti-MSC immune

response [29], which may cross-react with the donor kidney and increase the incidence of rejection and impact allograft survival in the long term. In a recent Phase I study, a single dose of $1.5–3 \times 10^6$/kg unmatched third party MSC were infused 3 ± 2 days after transplantation. Four out of 10 patients developed *de novo* donor-specific antibodies (dnDSA) against the MSC, one of which was also directed against the kidney graft. Renal function remained stable in patients during the study period leaving clinical relevance of the dnDSA unclear [30]. The Neptune study was set op to investigate the effect of third party allogeneic MSC after kidney transplantation with the aim to lower calcineurin inhibitor levels. To minimize the chance of anti-donor immune responses, a matching strategy was chosen to prevent repeated mismatches between the allogeneic MSC and the transplant kidney [31]. The results of this study are expected in the near future.

MSC mechanism of action

While clinical studies to the effects of MSC in organ transplant patients are ongoing, questions remain regarding their mode of action. It has been demonstrated that MSC interacts with a variety of immune and progenitor cells through the secretion of growth factors, cytokines and extracellular vesicles and induce beneficial immunomodulatory and regenerative effects [32,33]. It became clear some years ago, however, that intravenously infused MSC largely accumulate in the lungs due to size restriction of the pulmonary capillary network and have a short lifespan [34]. In recent years, important steps have been made in elucidating the mechanism of action of MSC therapy after intravenous infusion. It was demonstrated that during their brief presence MSC instruct host immune cells to adapt a regulatory function, and this effect persists after disappearance of the administered cells [35-37]. These regulatory immune cells migrate to other sites, such as the liver and lymph nodes but potentially also to sites of inflammation, where they may control inflammatory responses [38,39]. Thus, the short lifespan of MSC does not preclude a beneficial immunomodulatory effect of MSC in organ transplant patients. The implication of the short lifespan of MSC on their regenerative effects is yet unclear, but during their brief presence MSC may induce macrophages with regenerative properties [40,41]. An alternative to intravenous administration could be to deliver MSC directly to transplant organs on machine perfusion via the arterial flow.

Merging the fields of machine perfusion and MSC therapy

The first reports on the *ex vivo* delivery of MSC to porcine liver and kidney grafts have recently been published. In the liver, administered MSC show a wide range and patchy distribution [42] whereas in the kidney MSC localize specifically to the glomeruli [43,44]. The distribution pattern of MSC in liver and kidney is likely to depend on the size restriction of capillary networks in the organs, similar to the accumulation of MSC in the capillaries of the lungs after intravenous administration. This was demonstrated by arterial administration of dead MSC to kidneys on machine perfusion. While dead MSC are incapable of actively adhering to surfaces, the resulting distribution pattern was identical to that of living MSC [44]. When administering cells to organs on machine perfusion, it is important to use perfusion conditions that not only support the transplant organ, but also the administered cells. It appears that machine perfusion fluid has an effect on the adhesive properties of MSC in suspension, but that it does not affect the secretion of trophic factors by MSC [45]. Whether loading of transplant organs with MSC is beneficial for short-term and/or long-term organ function after transplantation, or whether other cell types at different doses would be more efficient will have to be determined in future studies.

Lessons learned from stem cell clinical trials outside the field of organ transplantation

Cellular therapies are notoriously difficult to initiate, in part due to regulatory requirements and the need for clinical grade cell production facilities and associated costs. By learning from previously approved trials, time and money might be saved and pitfalls related to cell production, logistics, inclusion criteria and clinical protocols avoided. While completed cell therapy trials in the field of organ transplantation with published results are relatively sparse, other fields have more extensive experience with MSC therapies. In the field of ischaemic heart disease, a number of trials have been finalized [46,47] or are ongoing [48,49]. Such trials teach us that it is advisable to keep the various processes in trials as simple as possible and choose clinically relevant inclusion criteria to increase clinical success rate. It is important to constantly follow-up on the regulation and collaborates with relevant partners with expertise in cell production, clinical trial set up

and regulatory aspects. When trials become more advanced, partnering with a commercial party may offer possibilities for progression towards efficacy testing and eventually the implementation of cellular therapies as an accessible treatment option for organ disease patients.

Corporate perspective on cell therapy

When cell therapy trials become larger and therapies start moving from the experimental setting to the therapeutic setting, academic centres are generally insufficiently equipped and funded to continue the research efforts without the help of a commercial partner. Athersys, amongst other clinical-stage biotechnology companies to collaborate with the academic sector, has developed a patented, allogeneic adult stem cell-derived off-the-shelf product for indications in areas of neurological, cardiovascular and inflammatory and immune disorders. Over the course of several years, the Athersys product has been investigated in a preclinical cardiac transplant model [50] and has been administered safely to patients receiving a liver transplant [51]. Outside the organ transplantation field, TiGenix-Takeda received marketing approval in the European Union in 2018 for the first allogeneic MSC product, and in the same year Mesoblast announced the positive results of its open-label Phase III trial in steroid-refractory acute GvHD, demonstrating that corporate involvement can bring cellular therapies closer to the clinic.

Decellularization and recellularization

Organs that are in a severe state of degeneration are unlikely to be responsive to reparative therapies through machine perfusion and adult stem cell therapies. For these organs, radical regenerative strategies are required. Recently, effective protocols for porcine and human livers decellularization have been developed that considerable shorten the duration of decellularization by increased pressure and flow without increased damage to the extracellular matrix [52]. The development of organ decellularization techniques has resulted in the accumulation of knowledge on the generation of acellular scaffolds for application in regenerative medicine, including for recellularization purposes. An interesting initiative in this field is the heterotopic implantation of decellularized hearts with the intent to allow the recipient body to repopulate the scaffold with endothelial and stromal cell types [53]. Other advances in this field are the generation of allogenic hydrogels for applications in tissue engineering, including 3D bioprinting [54].

The emergence of organoids for regenerative research

In recent years, considerable progress has been achieved in creating organ-like structures known as organoids from adult and pluripotent stem cells for virtually all types of tissue [55]. Kidney organoids with a surprising level of complexity can now be created within a few weeks from an undefined clump of pluripotent stem cells [56-59]. While initial work led to the generation of kidney organoids resembling first-trimester kidney tissue in structural organization and gene expression patterns, recent advances in culture protocols are driving differentiation further towards the second-trimester stadium [60]. Furthermore, implantation of organoids and subsequent vascularization in the host has been indicated to steer kidney organoid differentiation towards more maturity [61,62]. Importantly, it has been recently shown that the implantation of kidney organoids for a 5 days period leads to the organization of endogenous endothelial cells [60].

Other surrogates of organ-like microcultures are liver organoids. These can be generated from Leucine rich repeat-containing G protein-coupled receptor 5 ($Lgr5^{+}$) adult liver stem cells or from pluripotent stem cells and resemble the original liver epithelial architecture [63,64]. To implement the use of liver organoids for liver repair, new techniques are being explored including the use of decellularized liver to use as a scaffold for repopulation by organoid-derived cells [65]. Furthermore, improvements in the large-scale expansion of human liver organoids in oxygenated spinner flasks bring the application of these cells for whole-size graft repair in the clinical setting a step closer [66]. Advancements in upscaling have also been made for drug screening on cardiac organoids, where bioengineered human cardiac organoids find use for high-throughput testing of small molecules with pro-regenerative potential to stimulate cardiomyocyte proliferation [67] and long-term expansion methodology for airway organoids to allow disease modelling [68]. Other applications from the field of bioengineering include the implementation of organ-on-a-chip devices to promote kidney organoid vascularization [69] or the application of extrusion-based printing for the generation of kidney organoids aiming to provide solutions for current issues related to organoid inter-batch variability [70].

In the near term, organoids offer an unprecedented opportunity in organ transplantation research, including their application as models for studying organ disease, for (personalized) drug testing or for studying organ

development and physiology. In the future, it is anticipated that organoids will be applied as regenerative therapies. In the field of organ transplantation, this implies repair of aged and diseased transplant organs, and in the treatment of end-stage organ disease patients with an aim to repair organs and to delay or replace transplantation.

Ethical perspective

Developments in regenerative medicine raise many ethical issues including use of animal-testing in clinical research, research using human embryonic stem cells, foetal stems cells or discarded organs, issues with biobanking of 'live' material, informed consent for donation and use, commercialization of human cell-derived products, as well as research integrity and communication with the general public [71]. Human organoids may prove to be a useful alternative to animal models in clinical research and thus impact the ethical discourse on use of animal experiments in medical research. However, the human source of the stem cells used to develop organoids is of importance in this discussion. Similarly, patient or healthy volunteer-derived cellular therapies that replace current medications may increase the demand for human cells and tissues for research and therapies. Depending on the model and therapy, potential cellular sources include autologous tissues, placenta, umbilical cord or embryos. In particular, use of embryonic cells is controversial and views on this practice vary from protection of the human embryo leading to absolute prohibition to acceptance of usage given the potential for reducing human suffering. Ability to use this kind of cells for clinical research will depend on the context and relevant legal framework for that setting. A great step forward has been the generation of iPSC with properties of embryonic stem cells, which reduces the demand for embryonic stem cells. With regard to donor consent, research is needed to explore how donors perceive use of their cells to develop cellular therapies and what information and consenting process they find preferable. Issues of ownership and benefits from products and potential gains/profit from cell-derived therapies also require attention. While cell therapies present potential for innovation in care for patients with organ failure, their efficacy must first be proven in controlled trials [72]. This raises ethical concerns regarding the balance between risks and benefits for participants in (first-in-) human trials, patient selection, equality in access, risk-benefit assessment of treatment options, information provision, minimal requirements for informed consent, invasiveness and burden of the treatment, potential for adverse side-effects, long-term follow-up, and reimbursement. Ethical issues need to be considered for stem cells donors and participants in trials, as well as for researchers who may face pressure to publish, produce or commercialize their discoveries.

Conclusion

Regenerative medicine is a rapidly evolving tool that will affect the lives of the future organ transplant patient. Whilst regenerative medicine has the potential to make traditional organ transplantation redundant, multiple biological, technical, ethical and medical hurdles must be surmounted. Regenerative medicine will need to move to the forefront of transplantation research in the years to come to make its promises a reality.

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Conflicts of interest

The authors declare there are no conflicts of interest to report.

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