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POTENTIAL ALTERNATIVE APPROACHES TO XENOTRANSPLANTATION

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Abstract

There is an increasing worldwide shortage of organs and cells for transplantation in patients with end-stage organ failure or cellular dysfunction. This shortage could be resolved by the transplantation of organs or cells from pigs into humans. What competing approaches might provide support for the patient with end-stage organ or cell failure? Four main approaches are receiving increasing attention - (i) implantable mechanical devices, although these are currently limited almost entirely to devices aimed at supporting or replacing the heart, (ii) stem cell technology, at present directed mainly to replace absent or failing cells, but which is also fundamental to progress in (iii) tissue engineering and regenerative medicine, in which the ultimate aim is to replace an entire organ. A final novel potential approach is (iv) blastocyst complementation. These potential alternative approaches are briefly reviewed, and comments added on their current status and whether they are now (or will soon become) realistic alternative therapies to xenotransplantation.

Key Words

Blastocyst complementation

Pluripotent stem cells

Regenerative medicine

Tissue engineering

Total artificial heart

Ventricular assist device

Xenotransplantation

Abbreviations

ATIIC = alveolar epithelial type II cell

CRISPR/Cas9 = clustered regularly interspaced short palindromic repeat-associated system

ECM = extracellular matrix

ESC = embryonic stem cell

iPSC = induced pluripotent stem cell

PSC = pluripotent stem cell

TAH = total artificial heart

VAD = ventricular assist device

Introduction

Although the science is advancing rapidly, there remain some barriers to successful xenotransplantation (that are reviewed elsewhere in this issue). What alternative approaches are there? In other words, what competing approaches to xenotransplantation might provide support for the patient with end-stage organ or cell failure? Furthermore, how advanced is progress in these fields? Four main approaches are receiving increasing attention - (i) implantable mechanical devices, although these are currently limited almost entirely to devices aimed at supporting or replacing the heart, (ii) stem cell technology, at present directed mainly to replace absent or non-functioning cells, but which is also fundamental to progress in (iii) tissue engineering and regenerative medicine, in which the ultimate aim is to replace an entire organ, and (iv) blastocyst complementation.

Implantable mechanical devices

The Interagency Registry for Mechanical Assisted Circulatory Support (INTERMACS) now has data on >10,000 patients who have received some form of mechanical circulatory support during the past 8 years [1].

Ventricular assist devices

Patients in intractable heart failure can now be maintained by a left ventricular (or biventricular) assist device (LVAD, BVAD) in some cases for >2 years [1-3]. Whereas

originally they were inserted to support the patient until a heart from a deceased human donor became available (as a 'bridge' to transplant), they are increasingly inserted as 'destination' therapy, implying that no allotransplant will be carried out. In patients with continuous flow pumps, actuarial survival is approximately 80% at 1 year and 70% at 2 years [1-3], compared to survival following heart allotransplantation of approximately 85% and 80%, respectively. Mean hospital stay after implantation of a VAD is only 20 days [4].

Although these results are very encouraging, there are still problems associated with VAD support. The necessity for the patient to wear a portable power source or for the device to be connected to a stationary power source renders the quality of life less than optimal. In addition, in those being bridged to allotransplantation, sensitization to HLA antigens can develop (largely related to the need for blood transfusions), complicating the search for a suitable deceased donor heart [5]. Although data are very limited, this is not thought to be the case if bridging is by a pig xenograft [6].

There are several adverse events associated with long-term VAD support, e.g., thrombosis of the device [7], thromboembolism, and hemorrhage (primarily gastrointestinal) associated with the anticoagulation therapy that is essential [8]. Neurologic events remain significant [9,10]. Infectious complications related to the power lines that traverse the skin, thus providing a route of entry to microorganisms, remain problematic.

Total artificial hearts

Total replacement of the patient's heart with a mechanical device (total artificial heart, TAH) continues to be an option – or even essential - in specific groups of patients, for example, those with single-ventricle physiology [11], those who are not candidates for isolated left ventricular support by a VAD [12], and those with cardiac allograft failure [13]. Approximately 70% of patients with a TAH are successfully 'bridged' to cardiac allotransplantation, though the mortality on the device is approximately 25% [14]. Systemic infection (incidence of approximately 50%), driveline infection (25%), and thromboembolic/hemorrhagic events (30%) remain major complications.

Comment

Although in the first few clinical trials of cardiac xenotransplantation it is unrealistic to anticipate that the transplantation of hearts from pigs will be as successful as the current VADs, pig heart transplantation may well offer a viable alternative to a TAH [5]. Experience with VAD implantation extends back 45 years. It is likely that, when comparable experience is gained of cardiac xenotransplantation, the advantages of a natural, fully-implanted pig heart will outweigh those of a VAD.

Pluripotent stem cell therapy

Pluripotent stem cells (PSCs) have the capacity for self-renewal and are capable of differentiating into at least one, and sometimes many, specialized cell types.

Embryonic stem cells (ESCs) also have this capacity, but concern has been raised about the risk of teratoma formation and the ethics of using human embryos as a source of ESCs. Stem cell populations have also been identified in perinatal and adult tissues, including the amniotic fluid, placenta, bone marrow, and blood vessels. PSCs are also present in many tissues of adult animals and are important in tissue repair and homeostasis. Induced PSCs (iPSCs) can be generated through somatic cell reprogramming [15]. Because the PSCs could be of autologous origin, the immunological barriers to transplantation are relatively low [16]. PSCs therefore hold promise as a source for treating numerous disorders [17,18].

Pancreatic β cells

Pancreatic islet insulin-producing β cells have been derived from PSCs with the expression of different transcription factors [19,20]. Human pancreatic β cells may be transformed from stem or progenitor cells *in vitro*, cultured and expanded in number, and then transplanted into the patient [21]. If the stem or progenitor cells are derived from a specific patient, theoretically there may be no immune response to them after transplantation. However, it is uncertain whether (i) the transplantation of β cells alone (in the absence of the other cells in pancreatic islets, e.g., α cells) will be sufficient to maintain normoglycemia, and (ii) β cells derived from a patient with Type 1 diabetes will be susceptible after transplantation to damage by the autoantibodies that were a causative factor in the development of the original diabetes.

To our knowledge, no data have been reported demonstrating conclusively that PSC-derived β cells can maintain normoglycemia in a diabetic large animal model (in contrast to the results of pig islet xenotransplantation in nonhuman primates). Nevertheless, despite an absence of this evidence, a clinical trial of encapsulated laboratory-cultured β cell transplants (derived from PSCs) is underway in diabetic patients [22] (http://viacyte.com/).

Cardiomyocytes

PSCs can differentiate into cardiomyocytes and cardiac progenitors, which have been tested in animal models [23,24]. Transplanted mouse and guinea pig PSC-derived cardiac progenitors have improved the function of infarcted hearts [25]. Some *in vivo* repair of a damaged heart that has lost a significant percentage of its functioning myocytes, has been reported [26,27], but this approach is unlikely to be successful in patients in need of a heart transplant, where the myocardial damage is so extensive that 'repair' may not be sufficient. In these patients, nothing short of replacement of the entire organ is likely to improve its functional capacity.

Alveolar epithelial cells

Differentiation of PSCs into alveolar epithelial type II cells (ATIICs) has been developed by using a growth factor cocktail or a lung-specific cell-conditioned medium, but it remains unclear whether these derived ATIICs possess normal biological function [28,29]. However, this is a first step towards the generation of lung-specific PSC-derived ATIICs for exploring their clinical application in alveolar diseases.

Hepatocytes

Significant progress has been made in differentiating PSCs into hepatocyte-like cells, and may have clinical applications in the future [30-32].

Comment

The ability of PSCs to differentiate into cells that function and can replace absent or non-functioning cells remains limited. Nevertheless, further experience with PSC-derived β cells may enable patients to receive laboratory-cultured β cells that control diabetes, which might obviate the need for transplantation of pig islets. However, xenotransplantation of pig islets is currently in a more advanced state.

Tissue engineering/Regenerative medicine

Regenerative medicine is a relatively new field that involves the replacement or regeneration of cells, tissues, or whole organs to restore function [33]. As the complexity of the native tissues increases from flat structures (e.g., skin), to tubular structures (e.g., blood vessels), to hollow non-tubular organs (e.g., bladder), and to solid organs (e.g., heart), the complexity of tissue engineering increases. Nevertheless, tissue-engineered skin, cartilage, tracheas, blood vessels, urethras, bladders, and vaginas have been implanted in patients.

Decellularization of a solid organ, followed by recellularization of its remaining matrix with the potential patient's own cells is being explored [34]. Biologic scaffolds can be

composed of intact mammalian extracellular matrix (ECM), or individual components of ECM, such as collagen, laminin, or hyaluronan [35]. Such scaffold materials would ideally maintain the native ultrastructure and composition of the ECM. An implanted biologic scaffold can lead to constructive tissue remodeling, and this has been associated with significant clinical success.

There are successful clinical programs in which a relatively simple tissue, e.g., small intestine or bladder, derived from a pig, has been decellularized *ex vivo*, resulting in an ECM that is seeded with human cells, and then used to reconstruct a human tissue structure, e.g., bladder wall [36]. The trachea or bronchus [37-41] and esophagus [42,43], have been fashioned in the laboratory by taking a patient's bone marrow cells, expanding them in the laboratory, maturing them into chondrocytes and/or epithelial cells, and then seeding these on a decellularized segment of deceased human donor matrix, e.g., trachea. Recellularized airways have been transplanted into patients to replace deficient tracheas or bronchi [37,44]. (A decellularized pig tracheal matrix could equally be used, but may slightly increase the immune response.) No immunosuppressive therapy is required.

In an early study, the graft immediately provided the recipient with a functional airway, improved her quality of life, and had a normal appearance and mechanical properties at 4 months [37]. She was reported to remain well 5 years later [41]. The same result may possibly be achieved *in vivo*, with the patient's body being used as a bioreactor to

promote *in vivo* tissue replacement [38].

However, the barriers to generating a solid organ remain significant. Yagi et al decellularized adult pig livers [45], preserving the three-dimensional macrostructure. When seeded with isolated hepatocytes in a human-sized organ culture system, the hepatocytes engrafted and reorganized, and supported some liver-specific function, including albumin production, urea metabolism, and cytochrome P450 induction [34].

Kim et al have reviewed the feasibility of engineering a human-sized kidney [46]. Approaches to long-term implantation of engineered kidneys are under investigation using anti-thrombogenic strategies, such as functional reendothelialization of acellular kidney matrices, but several limitations need to be addressed before clinical translation is possible.

Bioprinting may enable the precise layer-by-layer organization of biological materials, biochemicals, and living cells into 3-dimensional structures [47], but bioprinting of solid organs will be more difficult [33].

Comment

It is realistic to anticipate that cell transplants may be feasible by a stem cell technology approach, but to generate an entire complex organ, such as a kidney or liver, particularly if vascular anastomoses are to be performed between organ and

recipient, is likely to require significant developments in the science and technology that may take several years [46]. As genetically-engineered pig organs are readily available today - and in the case of kidneys and hearts have functioned in nonhuman primates for several months or longer - it would seem that organ replacement through regenerative techniques is not an immediate competitor for xenotransplantation.

Blastocyst complementation

Generating a functional *human* organ from ESCs or iPSCs in a *pig* would be an ideal option since it would significantly reduce the immune response to the organ. 'Personalized' organs could be produced by using the patient's own iPSCs. This may possibly be achieved by knocking-out the genes for a specific organ, e.g., the lung, in the pig, and introducing human iPSCs that will generate a human lung in its place.

Chen et al. first reported the concept of "blastocyst complementation" in 1993 [48]. Nakauchi's group successfully generated almost totally iPSC-derived mouse kidneys in mice [49], as well as mouse-rat interspecies chimeras, i.e., the injected rat iPSC-derived cells formed almost the entire pancreas in the mice [50]. In addition, they demonstrated that blastocyst complementation is reproducible in pigs by using somatic cell nuclear transfer (cloning) technology [51]. New emerging genome-modifying technology, e.g., TALENs or the CRISPR/Cas9 system, has made the construction of organ-deficient pigs simpler and more convenient [52-54].

However, the ultimate goal of generating human organs in pigs remains in the future. First, it is not known whether human iPSCs or ESCs and pig blastocytes can form interspecies hybrids. The very considerable evolutionary distance between human and pig (about 100 million years) may prove a major obstacle in preventing critical signal transduction between pig and human cells during organ development, and may result in no formation or mal-formation of a human organ in a pig. Second, ethical issues have been raised since there is a possibility that human cells could be incorporated into the reproductive or central nervous systems of the pigs [55], although several options have been proposed to resolve such a concern [56].

Comment

Many obstacles remain to be overcome before blastocyst complementation becomes a clinical reality. Even if successful, it would allow for an organ to be produced for a single specific recipient on a case-by-case basis, but this would be costly and time-consuming. As the technology does not allow the repeated breeding of pigs with human organs, it would be unable to provide the thousands of organs and millions of cells required by the transplant community. It will therefore not be fully competitive with xenotransplantation.

Conclusions

Although the clinical results of implantable cardiac assist devices are currently far in advance of those of experimental pig heart transplantation in nonhuman primates,

there remain significant problems with this technology that may enable xenotransplantation ultimately to provide a better alternative. Differentiation and expansion of pancreatic β cells from human iPSCs may provide a source of cells that maintain normoglycemia after transplantation into diabetic patients, but islets from genetically-engineered pigs may provide a more reliable, less-costly, and equally (or more) effective therapy. The provision of thousands of organs by regenerative medicine techniques remains very much a future aim. Blastocyst complementation is an exciting approach but, even if successful, is unlikely to resolve the immense demand for organs and cells for clinical transplantation.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

LM, FC, YS, ZC, DKCC – all participated in review of the literature, writing of the manuscript, and final approval of the manuscript.

Guarantor

Lisha Mou

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Highlights

- Several technologies compete with xenotransplantation
- Mechanical devices are largely limited to cardiac support or replacement
- Pluripotent stem cells may ultimately cure diabetes
- Regenerative medicine can replace simple structures, but not yet solid organs
- Blastocyst complementation is unlikely to replace xenotransplantation