Recycling organs – growing tailor-made replacement kidneys

“...the idea of using the human kidney as platform for kidney bioengineering arose from the dramatic evidence that about 20% of the kidneys currently procured for transplant purposes are eventually discarded as unsuitable for transplantation.”

Keywords: cell • extracellular matrix • kidney • regenerative medicine

Regenerative medicine makes some big promises: to repair, regenerate, reprogram and engineer diseased or functionally impaired organs and tissues. Numerous strategies to achieve these extremely ambitious goals are in varying stages of development. At present, one seems to offer the quickest route to clinical application: the so called cell-on-scaffold seeding technology (CSST) [1,2], where cells are seeded on glycoproteic templates functioning as supporting scaffolding material.

CSST is based on the large body of evidence demonstrating that cells need their natural niche to be happy and exert their function [3]. In this process, auto- or hetero-logous cells are seeded on supporting scaffolds. These scaffolds are engineered to resemble the target organ in some cases, while others are derived from animal or human organs [4]. Using this method, more than 200 hundreds anatomically and functionally simple bio-artificial body parts were manufactured – in most cases *ex vivo* – by harnessing patients' own cells with custom built scaffolds [2]. In most circumstances, the seeded scaffolds were allowed to mature in bio-reactors for few days. Occasionally, they were implanted immediately after seeding [5]. In all cases, implantation was performed without reconnecting the vascular pedicle of the newly regenerated organ to the vasculature of the recipient

Despite these experiences have shown the immense potential of CSST to offer a new therapeutic approach to a myriad of clinical settings is immense, a lot of work remains to be done. Moreover, when the series reporting on the more than 200 implants are atten-

tively scrutinized, it is quite incontrovertible that follow ups are often short, most reports are anecdotal and lack in-depth discussion on the physiology of the bio-engineered organs as well as on complications and, more importantly, some clinical trials have been stopped due to high morbidity.

At Wake Forest, we are using CSST to bioengineer transplantable kidney organoids. For the purposes of our research, we use natural scaffolds derived from the extracellular matrix (ECM) of animal or human kidneys. The advantages of using natural scaffolds are numerous. Biomaterials from innate organs are inherently biocompatible; have their basic biochemistry (proteins and polysaccharides) and architecture sufficiently preserved; are able to drive differentiation of progenitor cells into organ-specific phenotypes; and retain an intact and patent vasculature. Moreover, ECM scaffolds are intrinsically functional when used as supporting scaffolding material. They serve as a reservoir of growth factors and cytokines, most of which remain after decellularization. These factors are responsible for their remarkable angiogenic properties including their ability to induce vessel formation in the chorioallantoic membrane, and for their striking ability to modulate the immune response. Elegant studies conducted by Paolo Cravedi at Mount Sinai, (NY, USA), have shown that ECM obtained from the human pancreas and kidney inhibits human naive CD4⁺ T-cell expansion in response to polyclonal stimuli by inducing their apoptosis and promoting their conversion into regulatory T cells [6]. While no technology can reproduce scaffolds with similar characteristics of any

Sheyna Gifford

Annenberg School for Communication & Journalism, University of Southern California, Los Angeles, CA 90007, USA

Joao Paulo Zambon

Department of Surgery, Section of Transplantation, Wake Forest School of Medicine, Wake Forest Baptist Hospital, Medical Center Blvd, Winston Salem, NC 27157, USA

Giuseppe Orlando

Author for correspondence:
Department of Surgery, Section of Transplantation, Wake Forest School of Medicine, Wake Forest Baptist Hospital, Medical Center Blvd, Winston Salem, NC 27157, USA
Tel.: +1 336 716 6903
Fax: +1 336 713 5055
gorlando@wakehealth.edu

given organ, these findings make ECM scaffolds far superior to their synthetic counterparts.

Current technology permits the production of ECM-based scaffolds from virtually all organs of mammals including rodents, pigs and humans. Our group initially developed a porcine model whose promising preliminary investigations lead us to scale up to the human model we use today. Our scaffolds are obtained mainly through the continuous perfusion of the organ of interest with a detergent-based solution, aiming at completely clearing the cellular compartment while preserving the ECM in terms of 3D architecture, molecular composition and physical characteristics. While no universally accepted method to decellularize organs exists, sodium dodecyl sulfate (SDS), Triton X100 and sodium deoxycholate are the most widely used. The preference for a specific detergent is determined by the organ's characteristics, in particular, its density, thickness and cellularity. Highly dense, thick and cellular organs like the kidney require an aggressive detergent; thus, SDS may be considered the gold standard. By contrast, less dense organs, like the small bowel or the pancreas, are more easily decellularized without need for SDS.

“We envision that, in the not too distant future, when cell-on-scaffold seeding technology reaches the bedside, a patient who is affected by end-stage renal disease may undergo robotic nephrectomy and have one native kidney processed to obtain autologous extracellular matrix scaffolds which will be reseeded with patient's own cells. The patient will then receive a new organ manufactured exclusively from his/her own ‘biomaterials’.”

A fair question to ask is: why use human kidneys for these sorts of investigations? In fact, the idea of using the human kidney as platform for kidney bioengineering arose from the dramatic evidence that about 20% of the kidneys currently procured for transplant purposes are eventually discarded as unsuitable for transplantation. We hypothesized that these roughly 2600 kidneys per year might become a valuable source of ECM scaffolds. We envision that, in the not too distant future, when CSST reaches the bedside, a patient who is affected by end-stage renal disease may undergo robotic nephrectomy and has one native kidney processed to obtain autologous ECM scaffolds which will be reseeded with patient's own cells. The patient will then receive a new organ manufactured exclusively from his/her own ‘biomaterials’. By this method – CSST using a patient's own failed organ – regenerative medicine may finally live up to its potential by addressing the two most urgent needs of modern organ trans-

plantation: organ-on-demand, through the identification of a potentially inexhaustible source of organs, and immunosuppression-free transplantation, ruling out the need for lifelong antirejection therapy.

Before we arrive at that brilliant solution to two of the most pressing problems in transplant medicine, many questions will have to be answered. For one, we must address the fact that in the ESRD patients the ECM of the scaffolds is partially diseased. In fact, the kidneys used to produce the scaffolds in question are discarded for moderate to severe biopsy-proven chronic disease (i.e., scarring in the form of glomerulosclerosis and/or tubular atrophy) or acute (massive necrosis) damage, or, more rarely, for excessive cold ischemia time or anatomical anomalies. However, the presence of scarring tissue *per se* may not be an issue as very interesting experimental and clinical data from settings not limited to nephrology show that the human body has the ability to reverse fibrosis [7–9]. Moreover, preliminary data from our group show that when multipotent cells are seeded on discs of the ECM scaffolds in question, cells do, as expected, attach, migrate and function well. Post-re-seeding, these cells have demonstrated their ability to remodel the matrix and mount an inflammatory response in the context of which a strong angiogenesis is observed [10,11].

Studies are needed to understand whether our ECM scaffolds from discarded organs truly mimic the natural niche and, if they do, successful seeding of cells should be followed by appropriate attachment, proliferation and function. However, even after the functional units of the kidney are reseeded, will an organ regenerated on a partially diseased matrix successfully sustain a patient's kidney function in the long-term? This brings us to a critical, yet unanswered, question. We speculate that ECM scaffolds from discarded kidneys may still provide an adequate support to the cells as well as a niche-like environment, as long as areas of normal ECM exist. Taking into account the fact that the regenerated organs are seeded onto diseased matrices, is the volume produced during the growth of these bioengineered kidney sufficient to provide long-term function to the patient?

For CSST, and regenerative medicine as a whole, the preliminary data are promising. However, the functioning reseeded of the cellular compartment of an anatomically and physiologically complex organ like the kidney remains an elusive goal, regardless of the scaffold used. To date, research has shown that the key components to ensure the success of CSST, and similar technologies, include: a comprehensive characterization of the ECM of the human kidney; understanding the ECM cells interactions; proven strategies for revascularization of the ECM scaffolds;

identification of most of the appropriate cells for recellularization of the organ's parenchyma; assessment of the immunogenicity of ECM scaffolds before and after recellularization; feasibility of transplanting the constructs and its ability to sustain physiologic blood pressure and remain free of clotting; designing of physiological biomimetic bioreactors; and assessment of the viability and function *in vitro* and *in vivo* of the bioengineered organs.

A lot of work remains to be done. It is also strikingly clear that the ultimate goal will be achieved only if and when forces from multiple yet complementary fields and groups will be joined. Equally impor-

tant will be for researchers not to commit the error to attempt translation without adequate scientific foundations.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- 1 Salvatori M, Peloso A, Katari R *et al.* Semi-xenotransplantation: the regenerative medicine-based approach to immunosuppression-free transplantation and to meet the organ demand. *Xenotransplantation* 22, 1–6 (2015).
- 2 Orlando G, Soker S, Stratta RJ. Organ bioengineering and regeneration as the new Holy Grail for organ transplantation. *Ann. Surg.* 258, 221–232 (2013).
- 3 Hynes RO. The extracellular matrix: not just pretty fibrils. *Science* 326, 1216–1219 (2009).
- 4 Badylak SF, Weiss DJ, Caplan A, Macchiarini P. Engineered whole organs and complex tissues. *Lancet* 379, 943–952 (2012).
- 5 Hamilton NJ, Kanani M, Roebuck DJ *et al.* Tissue-engineered tracheal replacement in a child: a 4-year follow-up study. *Am. J. Transplant* doi:10.1111/ajt.13318 (2015) (Epub ahead of print).
- 6 Peloso A, Urbani L, Katari R *et al.* The human pancreas as a source of pro-tolerogenic extracellular matrix scaffold for a new generation bio-artificial endocrine pancreas. *Ann. surg.* (2015) (In Press).
- 7 Remuzzi A, Gagliardini E, Sangalli F *et al.* ACE inhibition reduces glomerulosclerosis and regenerates glomerular tissue in a model of progressive renal disease. *Kidney Int.* 69, 1124–1130 (2006).
- 8 Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J. Clin. Invest.* 116, 288–296 (2006).
- 9 Hammel P, Couvelard A, O'Toole D *et al.* Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. *N. Engl. J. Med.* 344, 418–423 (2001).
- 10 Peloso A, Petrosyan A, Da Sacco S *et al.* Renal extracellular matrix scaffolds from discarded kidneys maintain glomerular morphometry and vascular resilience and retains critical growth factors. *Transplantation* 99(9), 1807–1816 (2015).
- 11 Petrosyan A, Orlando G, Peloso A *et al.* Understanding the bioactivity of stem cells seeded on ECM produced from discarded human kidneys. *Cell* 3(1), e1401 (2015).