Applications of regenerative medicine in organ transplantation

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ABSTRACT

A worldwide shortage of organs for clinical implantation establishes the need to bring forward and test new technologies that will help in solving the problem. The concepts of regenerative medicine hold the potential for augmenting organ function or repairing damaged organ or allowing regeneration of deteriorated organs and tissue. Researchers are exploring possible regenerative medicine applications in organ transplantation so that coming together of the two fields can benefit each other. The present review discusses the strategies that are being implemented to regenerate or bio-engineer human organs for clinical purposes. It also highlights the limitations of the regenerative medicine that needs to be addressed to explore full potential of the field. A web-based research on MEDLINE was done using keywords "regenerative medicine," "tissue-engineering," "bio-engineered organs," "decellularized scaffold" and "three-dimensional printing." This review screened about 170 articles to get the desired knowledge update.

KEY WORDS: Bioartificial organs, regeneration, regenerative medicine, tissue-engineering

Organ replacement field is now solidly entrenched in modern medical therapy. Numerous patients have received new kidneys, livers, and hearts. Other organs (lung, pancreas, and intestine) are also routinely transplanted, although in less numbers. Organ replacement therapy have resulted in more active, productive, and meaningful lives of recipients of all organs.[1,2] Although a success, but transplantation raises a number of bioethical issues,[3] including obtaining consent and payment for an organ to be transplanted.[4,5] Other ethical issues include transplantation tourism and the socio-economic context in which organ harvesting or transplantation may occur. Limited supply of organs is causing serious crime of organ trafficking. Compensated donation (donors get money or other compensation in exchange for their organs) is common in Pakistan, India, China, and some other countries and helps in driving medical tourism.[6] The gap between organ need and organ availability continues to widen despite very substantial public education efforts on organ donation. This background needs to be dealt with immediate attention and demands for finding an alternative source of organ transplantation.

Groundbreaking achievements in regenerative medicine have generated interest in transplant researchers and they are exploring possible regenerative medicine applications in organ transplantation so that coming together of the two fields can benefit one another. New technologies consisting of stem cells and tissue-engineering have potential for augmenting organ function or repairing damaged organ. If explored to their full potential organ bio-engineering and regeneration technologies hold the promise to address two most urgent needs in organ transplantation, namely, the identification of a new, potentially inexhaustible source of organs and immunosuppression-free transplantation of tissues and organs.[7] The present review aims to illustrate the strategies that are being implemented to regenerate or bio-engineer human organs for clinical purposes.

A web-based research on MEDLINE was done for collecting

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data for the review. To limit our research to relevant articles, the search was filtered using terms Review, published in the last 10 years in MEDLINE journals. Keywords used for research were "regenerative medicine," "tissue-engineering," "bio-engineered organs," "decellularized scaffold" and "three-dimensional (3D) printing." This review screened about 170 articles to get the desired knowledge update. Important cross references were also studied and only relevant information was compiled.

Techniques for Organ Regeneration used in Regenerative Medicine

Regenerative medicine is the "process of replacing or regenerating human cells, tissues or organs to restore or establish normal function."[8] It helps in regenerating damaged tissues and organs in the body by replacing damaged tissue or healing previously irreparable tissues or organs. The various techniques that are being used for organ regeneration include cell transplantation/cell therapies; organ generation using a single adult tissue stem cell, a blastocyst complementation system coupled with a specific stem cell niche, decellularization and recellularization of bio-scaffold, *in vitro* grown organs and tissues through concepts of tissue-engineering, organ printing, and xenotransplantation.

Cell therapy

Although, the goal of regenerating a functionally complex organ is still far-off and controversial, cell transplantation/cell therapy is a practical procedure. Cell therapy can be defined as a therapy in which cellular material is injected into a patient.[9] There are two divisions of cell therapy. The first category includes transplantation of human cells from a donor to a patient. It has strong prospects for future growth. Therapeutic applications include neural stem cell therapy, mesenchymal stem cells (MSCs) therapy and others such as hematopoietic stem cell transplantation. Such therapies have shown promising results in cases of osteogenesis imperfecta,[10] Hurler's Syndrome patients,[11] myeloid malignancies[12] and other blood cell diseases. A milestone was hit in the field of neural stem cell therapy in 2009 when the US food and drug administration granted permission to the company Geron to initiate the world's first human clinical trial of an embryonic stem cell-based therapy for acute spinal cord injury. Initial preclinical testing showed the method was safe and efficient in improving locomotor skills in animal models.[13] However, Geron discontinued the clinical trials because of financial difficulties. Recently, functional regeneration of supraspinal connections in a patient with transected spinal cord was achieved following transplantation of autologous bulbar olfactory ensheathing cells with peripheral nerve bridging.[14] Cell transplantation method for replacing myocardium and clinical trials for cardiac cell therapy are being prioritized and funded in multiple countries.[15,16] Cell therapy can be performed with much less risk to the patient. Furthermore, it can also be applied to patients who are severely ill and would not be able to tolerate organ transplantation. This approach holds a promising future, but is challenged by shortage of donor cells, poor cell survival as well as by low transplant efficiency and may lack true regeneration. The second category includes the practice of injecting animal materials to cure disease. This practice, lacks any medical evidence of effectiveness and can have very serious consequences.[9]

Generation of a functional organ from a single adult tissue stem cell

This approach involves the generation of an entire organ from a single stem cell purified from the tissue. Utilizing this concept successful generation of secretory mammary glands was achieved by transplanting single stem cells isolated from adult mouse mammary glands into the fat-pad in mice.[17,18] Similarly, using a colony-formation *in vivo* assay and an *in vivo* renal capsule transplantation approach, Leong *et al*., have also reported that a single stem cell isolated from the adult mouse prostate epithelium has the capacity to generate a functional prostate.[19]

Generation of organs using a blastocyst complementation system

Originally reported by Chen *et al*.,[20] this approach can be used to create chimeric animals that have organs belonging to another species. This approach is based on the concept that a missing organ can be generated from exogenous cells when functionally normal pluripotent cells chimerize a cloned dysorganogenetic embryo. The concept was successfully applied to generate pluripotent stem cell (PSC) – derived rat pancreas and kidney.[21,22] This blastocyst complementation system may provide a novel approach for organ supply by generating specific human organs from various PSCs injected into xenogeneic sources.

Decellularization of matrix bioscaffolds and recellularization with stem cells

In this decellularization of tissues and organs is done to generate acellular biologic scaffolds with preserved extracellular matrix (ECM) that can be recellularized with selected progenitor cell populations. Decellularized organs provide the ideal transplantable scaffold with all the necessary microstructure and extracellular cues for cell attachment, cell differentiation, cell vascularization, and cell function. In 2008, Macchiarini *et al*. performed the first adult stem cell grown trachea transplant[23] obtained by decellularizing deceased donor trachea leaving behind connective tissue scaffold which was then re-seeded with cells from the recipient (chondrocytes on the outer surface and epithelial cells on the inner surface). This approach has been extended to treat patients with tracheal cancer. Successful seeding of decellularized mouse heart as scaffolds with induced pluripotent stem cell (iPSC)-derived cardiovascular progenitor cells has also been reported.[24] One of the first reports to successfully recellularize decellularized scaffolds with human liver cells was by Baptista *et al*., who demonstrated the potential for the colonization of human hepatocyte progenitors on a decellularized liver matrix.[25] Literature shows successful utilization of decellularized scaffolds

for tissue-engineering of lung,[26] urinary bladder,[27] urethra,[28] and blood vessel.[29]

Decellularization-recellularization technique can also be utilized for organ engineering for digestive tract regeneration and replacement. Isch *et al*.,[30] used decellularized human skin for patch esophagoplasty and reported successful healing of epithelial injury of the esophagus. Badylak *et al*.,[31] used decellularized xenogeneic urinary bladder as a scaffold re-seeded with autologous muscle cells, resulting in functional reconstruction of the esophageal wall clinically. Mertsching *et al*.,[32] investigated a transplantable intestine vascularized with human endothelial cells.

Extracellular matrix scaffolds are currently used for arterial grafts, heart valves, dura mater grafts, skin reconstruction, urinary tract reconstitution, and orthopedic applications. Some of the scaffolds are available commercially including: Porcine heart valve (Hancock II®), bovine pericardium heart valve (Perimount Magna®), human heart valve (Synegraft®), human dermis (Alloderm®), porcine small intestinal scaffold (OaSIS®), and decellularized bone (Allograft c-ring®).[33]

Tissue-engineering

Concurrent growth in the fields of material bio-engineering and cell biology has led to the possibility to grow tissue-engineered organs. Bio-engineered organs have advantage of not prone to transplant rejection as they rely on a patient's own cells.

Tissue-engineered small intestine

It offers a potential autologous therapy that avoids the problems of donor graft supply for intestinal transplant and long-term immunosuppression. Originally described by Vacanti *et al*., tissue-engineered small intestine (TESI) has been produced by *in vivo* implantation of organoid units, which are multicellular clusters of epithelium and mesenchyme harvested from the native intestine. These organoid units are seeded onto a scaffold and implanted into the omentum of the host resulting in TESI.[34] TESI exactly recapitulates histology of the native intestine showing all four epithelial lineages in conjunction with lamina propria, nerve elements, and muscularis mucosa along with enteric neuronal plexuses. However, it did not regenerate the alignment of the circular and longitudinal smooth muscle that is crucial for generating appropriate force and motility to facilitate nutrient absorption.[35]

Bladder

Various natural and synthetic biomaterials such as gelatin sponge, plastic mold, lyophilized human dura, small intestinal submucosa etc., have been used for urinary bladder regeneration with a wide range of outcomes.[36] An alternative emerging method involves growing a bladder from autologous stem cells seeded on a bladder-shaped scaffold.[37] In 2006, the first publication of experimental transplantation of bio-engineered bladders appeared in The Lancet.[38] Researchers grew urothelial and muscle cells in culture and seeded these cells on a biodegradable bladder-shaped scaffold comprising of collagen or composite of collagen and polyglycolic acid. They successfully produced bio-engineered bladders which were then implanted back into the patients' bodies.

Trachea

In June 2011, Macchiarini *et al*., successfully implanted an artificial trachea in a 36-year-old patient with late-stage tracheal cancer. Stem cells taken from the patient's hip were treated with growth factors and incubated on a plastic replica of his natural trachea.[24] The advantage of using an artificial structure is that the donor is not required, and trachea can be replaced within days.

Heart

Tissue-like cellular patches have been developed by using biomaterials acting as a delivery platform for the cells to improve the efficiency of stem cell therapies. Porous biomaterials, such as alginate or poly-glycolide-co-lactide like polymers, have also been tested as cell scaffolds with human embryonic stem cell-derived cardiomyocytes.[39] New strategies like micro templating or electrospinning have also been incorporated to create scaffolds to control a homogenous seeding of the cells allowing an organized and aligned distribution.[40] Scaffold-free cell sheet-based tissue-engineering has been introduced by Shimizu *et al*.,[41] for construction of 3D tissue-like structure. Gene therapy that includes direct introduction of transgenes into the vasculature or myocardium to control the symptoms of diseases and might also reverse the pathological conditions is also being studied as a potential treatment option.[42] Alternatively reprogramming of endogenous nonmyocytes into cardiomyocytes are also being explored but require validation before clinical trials.[43]

Kidney

Bio-engineering has led to successful production of a renal assist device with human cells and was successfully used on humans in an extracorporeal setting.[44,45] In this approach, renal parenchymal cells are harvested and seeded onto the internal surface of hemodialysis hollow fibers to mimic the resorptive capacity of renal tubules. This bioartificial approach using human renal epithelial cells now is referred as renal bio replacement therapy and has been used in phase I/II clinical trials.[45] It is now being moved into a phase III clinical trial. To address the adverse effects of immunosuppressants, Lanza *et al*.,[46] successfully used nuclear transplantation for renal regeneration. Recently researchers have pinpointed the precise cellular signaling responsible for kidney regeneration and exposing the multi-layered nature of kidney growth.[47] This research opens the pathway to achieve human kidney regeneration.

Liver

The "cell sheet" technology developed by Okano *et al*.,[48] has

the potential for successful clinical translation. It consists of stacking of upto four hepatocyte cell sheets that can be readily engrafted and provide a specific metabolic relief to the recipient.[49] Until recently, it was generally believed that liver organogenesis could not be reproduced *in vitro*, but a major breakthrough in the production of bio-engineered livers was registered with a study by Takabe *et al*.,[50] in which they were able to construct a liver in a petri dish and hepatic endoderm cells (HEs) derived from human iPSCs (iPSC-HEs) were cultured with human umbilical vein endothelial cells and human MSCs. Such bio-engineered livers could also be useful for evaluating the safety of new drugs.

Ovaries

A precursory human ovary has been developed[51] with self-assembled microtissues created using novel 3D petri dish technology with intention of studying *in vitro* maturation of immature oocytes and the development of a system to study the effect of environmental toxins on folliculogenesis. Researchers in the US have bio-engineered an artificial ovary that makes sex hormones in the same proportions as a healthy one. The bio-engineered ovary shows sustained released of sex hormones estrogen and progesterone *in vitro*. Such bio-engineered ovaries may provide a more natural option for women than hormone replacement therapy.[52]

Thymus

Researchers succeeded in rejuvenating a fully involute aged thymus.[53] Rejuvenated thymus closely resembled juvenile thymus in terms of architecture and gene expression profile. The study establishes that upregulation of a single transcription factor can substantially reverse age-related thymic involution. The breakthrough can have a broad impact in areas of regenerative medicine.

Ear

In 2006, Wada *et al*., successfully reconstructed inner ear tympanic cavity and mastoid cavity in rats using a biodegradable collagen scaffold. Outer ear has also been successfully regrown controlling the shape of the ear through shaping of the scaffold structure.[54]

Tissue-engineered skin

An artificial complete skin (dermis and epidermis) model has been developed for the treatment of severe epithelial injuries.[55]

Organ printing

Organ printing is a new emerging technology which represents an alternative to classic biodegradable solid scaffold-based approaches in tissue-engineering. Organ printing can be defined as layer-by-layer additive robotic biofabrication of 3D functional living macrotissues and organ constructs using tissue spheroids as building blocks.[56] Organ printing involves three sequential steps: Preprocessing or development of blueprints for organs in which digitized image reconstruction of a natural organ or tissue is obtained; processing in which actual organ printing is done by layer-by-layer placement of cells or cell aggregates into a 3D environment; and post-processing involving perfusion of printed organ and accelerated organ maturation.[57] It uses the principle of cellular self-assembly into tissues.[58] Organovo company was the first to commercialize 3D bioprinting technology[59] and utilizes NovoGen MMX Bioprinter manufactured by Invetech partnered with Organovo, which is capable of printing heart tissue, blood vessels, skin tissue, etc., Bioprinting technology is also being used to produce soft tissues and artificial bones for eventual use in reconstructive surgery.[60] Several studies have demonstrated the capacity of 3D bioprinting for the generation of 3D structures for various tissue regeneration applications, including skin,[61] bone tissue constructs[62] and cartilaginous structures.[63] Recently a bioresorbable customized tracheal splint was fabricated with the use of laser-based 3D printing, to treat a life-threatening condition in an infant.[64] The feasibility of building bioartificial blood vessel-like constructs for research and potential clinical uses has also been demonstrated by Bioprinting vessel-like constructs using hyaluronan hydrogels cross-linked with tetrahedral polyethylene glycol tetraacrylates.[65] Duan *et al*. fabricated mechanically living tri-leaflet heart valves using 3D printing and multiple valve cell populations.[66] Currently, scientists are working on developing kidneys, bladders, and hearts using this technique. Though they have not yet been able to recreate a fully functional organ, they have succeeded in creating small organ models of human heart and kidney. The major drawback of these organ models is that their lifespan is limited to days rather than years.[67]

Regenerative Applications in Dentistry

Although, in recent years regenerative medicine has undergone significant advancement but dentistry is not far behind. Regenerative Dentistry promises number of clinical benefits that include strategies to repair teeth and restore teeth after carious damage, resolve intraosseous periodontal defects and advanced grafting procedures for maxilla and mandible.[68] Most research is directed towards dentin regeneration, pulp regeneration, periodontal regeneration, restoring the resorbed root, and repairing the root perforations. In addition, tissue-engineering applications to promote healing of oral wounds/ulcers as well as gene-transfer methods to manipulate salivary proteins and oral microbial colonization patterns are being studied.[69] Wei *et al*., successfully regenerated a functional bio-root structure for artificial crown restoration by using allogenic dental stem cells.[70] Successfully functioning tooth in a mouse achieved through the transplantation of bio-engineered tooth germ into the alveolar bone have also been reported.[71] The feasibility of dental stem cells of American alligators to regenerate teeth in humans is also being studied.[72] Nondental stem cells for dental applications have also been explored. Cai *et al*., reported a method for growing teeth from stem cells obtained in urine.[73] Whole tooth regeneration to replace the traditional dental implants is also in pipeline.[74] Various regenerative approaches used in endodontics are root canal revascularization, postnatal stem cell therapy, scaffold

implantation, injectable scaffold delivery, pulp implantation, 3D cell printing, and gene therapy.[75] Of all these, only root canal revascularization approach is clinically feasible while rest others exist in research fields.[76] With new discoveries, innovative ideas, and high-quality research, regenerative therapies have the potential to revolutionize dentistry.

Limitations of Organ Bio-Engineering

Until date, all the successful implanted bio-engineered organs are hollow organs, whereas the bio-engineering of modular organs such as cardiac, renal, hepatic, and pancreatic is still far from the realm of possibility.[7] There are certain weak links that need to be addressed for achieving success in organ bio-engineering and regeneration. The reported clinical implantations of bio-engineered organs are with short follow-up and insufficient discussion of complications and limitations that may occur with time. The iPS cell technology has great potential, but it is important to evaluate the methodologies for iPS cell generation for their safety and efficacy.[77] The present technology does not seem to produce adequate bioreactors to mimic *in vivo* conditions, i.e., temperature, nutrient, and oxygen concentration required for the maturation of bio-engineered organ.[7] Vascularization of implanted bio-engineered constructs is required. Decellularizing process to produce accellular scaffold subjects the tissue to number of detrimental factors that result in disruption of architecture and potential loss of surface structure and composition resulting in reduced mechanical properties as compared with those of the normal native organs.[78] Thus, optimal methods of decellularization should be employed. Furthermore, a wide range of non-ECM proteins are retained in decellularized scaffolds with the current techniques of which several retained proteins (e.g. histones) are known to be immunogenic.[79]

Seeding techniques, number and types of cells required for seeding, and composition and architecture of biomaterials are key factors for success in organ bio-engineering and regeneration, and lack or impairment of just one of these factors may lead to failure, regardless of whether all other factors are appropriate.

Conclusion

The march of regenerative technology has changed our concepts of organ transplantation. Although we are not theoretically far-off from beginning to understand human regeneration, it still remains a science of future due to ethical limitations. Regardless of whether regenerative organ therapy succeeds, we believe it may generate new knowledge and new visions about how organs may be replaced in the future. It is the shared hope that regenerative medicine may one day augment organ transplantation by developing a new source of organs or potentially rehabilitating those that are not transplantable.

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Conflict of interest

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