

Manifestation of Novel Social Challenges of the European Union in the Teaching Material of Medical Biotechnology Master's Programmes at the University of Pécs and at the University of Debrecen

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Transdifferentiation and regenerative medicine – Lecture 12

KIDNEY REGENERATION



The kidney

- The kidney participates in blood filtration, but is also involved in hormone secretion and bone metabolism.
- The kidney is considered as one of the highly differentiated organs of the body.
- The kidney is regarded as an organ incapable of regeneration, since no new nephrons appear after 36 weeks of gestation in humans as a result of the exhaustion of progenitor mesenchyme.
- However, accumulating evidence show that kidney also undergoes continuous slow cellular turnover for tissue maintenance and cell replacement after injury.
- The lower proliferative fate of the kidney may be due to gene programming of its constituent cells.

Renal disease

- Numbers of renal disease patients are increasing worldwide, so there is a strong requirement for traditional renal replacement therapy along with alternative approaches.
- Acute kidney failure (AKF) means a rapid decrease of renal function with increased creatinine level. Causes of AKF are inadequate renal perfusion, hemorrhage and loss of intravascular fluid, low cardiac output, low systemic vascular resistance, acute tubular injury, glomerulonephritis.
- Chronic kidney disease (CKD) is characterized by a long-standing, progressive deterioration of renal function. These symptoms develop slowly leading to End Stage Renal Disease (ESRD). The most common cause of CKD is diabetic nephropathy, followed by hypertensive nephroangiosclerosis and various glomerulopathies, such as IgA nephropathy.

Renal replacement therapy I/a

Hemodialysis:

- A long term therapy approach with recent developments such as decreased membrane thickness with the so called Human Nephron Filter (HNF).
- The HNF consists of two membranes that operate in series within one cartridge.
- The first membrane is called the G membrane and is analogous with the glomerular membrane in the nephron. It mimics the functions of the glomerulus by using convective transport to generate plasma ultrafiltrate that contains solutes that approach the molecular weight of albumin.
- The second membrane is called the T membrane and mimics the functions of the tubule. It is molecularly engineered and selectively reclaims convectively the designated solutes to maintain homeostasis.
- No dialysate is used in the system.

Renal replacement therapy I/b

Hemodialysis:

- The G membrane discriminates between solutes on the basis of molecular size. The ultrafiltrate formed after blood passes through the G membrane contains both desirable and undesirable solutes.
- The ultrafiltrate passes over the T membrane, which reclaims most of the desirable solutes and rejects the undesirable ones. The T membrane is able to differentiate because each of its pores is a designed discriminator and is made up of multiple unique pores. The pores were designed to reabsorb all of the desirable solutes and reject the undesirable ones. Pores with similar radii can be designed to have very different and selective transport properties.

Renal replacement therapy II

Bioartificial kidney:

- In order to overcome the lack of regulatory, metabolic, and endocrine function in current dialysis systems, a combination of living renal cells and polymeric scaffolds has been proposed (Phase I/II trials in human).
- This technique depends on the ability to isolate and grow adult tubular cells in culture. These cells are subsequently grown along the inner surface of the fibers of the standard hemofiltration cartridge. This tubule cell cartridge is then placed in series with a conventional hemofilter, constituting a bioartificial kidney called a renal assist device.
- *In vitro* and *ex vivo* tests of renal assist devices have been conducted of animals and critically ill patients, with promising results that include better cardiovascular and hemodynamic parameters.

Renal replacement therapy III

Wearable kidney:

- Uses a sorbent system that regenerates dialysate, reminiscent of the peritoneal-based wearable system.
- For up to 10 h a day was well accepted by patients which may improve compliance compared to current dialysis systems.
- On average, 99.8 ± 63.1 mg of β 2-microglobulin was removed, with a mean clearance of 11.3 ± 2.3 ml/min, and an average of 445.2 ± 326 mg of phosphate was removed, with mean plasma phosphate clearance of 21.7 ± 4.5 ml/min. These clearances compared favorably with mean urea and creatinine plasma clearances (21.8 ± 1.6 and 20.0 ± 0.8 ml/min, respectively).

Tissue engineering of the kidney

Partial or complete *de novo* reconstruction of the whole organ is being investigated for their potential in clinical applications.

Ex novo kidney reconstruction:

- Efforts are made to develop an *in vitro* functional tissue/organ that resembles the native physiology and morphology of the kidney and can be readily incorporated *in vivo* with low immunogenicity.
- *In vitro* scaffold with various cells implanted to build a 3D structure of the kidney.

Embryonic kidney culture for transplantation

- As appropriate growth factors are added to an *in vitro* system, a branching ureteric bud (the progenitor tissue from which the renal collecting system is derived) can be successfully cultured and maintained, which subsequently can induce the growth and differentiation of the metanephric mesenchyme (MM, the tissue from which nephron is derived) into a primordial kidney structure ready for transplantation.
- It is possible to transplant these embryonic metanephri into an *in vivo* model and demonstrate that these primordial kidneys are able to survive, develop and also secrete concentrated filtrate.

Artificial scaffolding in kidney transplantation I

- Tissue Engineering is based on the concept of using a scaffold (natural or synthetic) that can work as a “skeleton” in which to seed different cell types and provide the 3D structure for seeded cells to proliferate and differentiate leading to a functional organ.
- The scaffolds may consist of biologic materials (carbohydrates, proteins, and peptides); synthetic materials such as polymeric, ceramic and metallic materials; or tissue from human or animal sources.
- For *ex novo* kidney reconstruction, use of collagen and matrigel scaffolds, thermo responsive polymers, hollow fibers or pre-molded biodegradable polymers has been proposed, but these compounds are not able to replicate the precise spatial organization of complex structures.

Artificial scaffolding in kidney transplantation II

- Successful combination of scaffolds and living cells are extremely challenging.
- Primary kidney cells can be successfully seeded in a collagen based culture system and renal progenitor cells can be seeded on artificial polyester interstitium.
- Glomerular epithelial and mesangial cells showed tissue reconstruction and polarity *in vitro*.
- By nuclear transfer somatically identical renal cells were produced in bovine. After retrieval, cells were characterized for renal markers (aquaporin 1, AQP2, synaptopodin). These renal cells were placed onto collagen coated cylindrical polycarbonate membranes and transplanted *in vivo* into the original animal. Within 12 weeks, it was shown that the transplanted cells were capable of secreting urinary fluid.

Kidney regeneration *de novo* using blastocysts

- Injection of normal ES cells into the blastocysts of recombination-activating gene 2 (RAG-2)-deficient mice, which have no mature lymphoid cells, could generate somatic chimeras with ES cell derived mature B and T cells.
- Injection of wild-type ES cells into the blastocysts of Sall1-null mice, which lack kidneys, generated metanephroi were composed exclusively of ES cell-derived differentiated cells.
- Moreover, iPS cell-derived progeny can occupy that niche and can compensate developmentally for the missing contents of the niche, forming a complicated organ composed almost entirely of cells derived from donor iPS cells.

Kidney regeneration *de novo* using xenoembryos I

- During development of the metanephros, the metanephric mesenchyme initially forms from the caudal portion of the nephrogenic cord and secretes glial cell line derived neurotrophic factor (GDNF), which induces the nearby Wolffian duct to produce a ureteric bud. When this epithelial mesenchymal induction to occur, GDNF must interact with its receptor, c-ret, which is expressed in the Wolffian duct.
- GDNF-expressing hMSCs may differentiate into kidney structures if positioned at the budding site and stimulated by numerous factors spatially and temporally identical to those found in the developmental milieu.

Kidney regeneration *de novo* using xenoembryos II

- Rat embryos were isolated from the mother before budding and were grown in a culture bottle until the formation of a rudimentary kidney so that it could be further developed by organ culture *in vitro*.
- hMSCs were microinjected at the site of budding.
- Reporter gene positive cells were scattered throughout the rudimentary metanephros and were morphologically identical to tubular epithelial cells, interstitial cells, and glomerular epithelial cells.
- These data demonstrated that using a xenobiotic developmental process for growing embryos allows endogenous hMSCs to undergo an epithelial conversion and be transformed into an orchestrated nephron consisting of glomerular epithelial cells (podocytes) and tubular epithelial cells that are linked. hMSCs can also differentiate into renal stroma after renal development.

Kidney regeneration *de novo* using xenoembryos III

- The next step in the successful development of an artificial kidney *de novo* is the urine production.
- The kidney formed needs to have the vascular system of the recipient. The metanephros can grow and differentiate into a functional renal unit with integration of recipient vessels if it is implanted into the omentum.
- The vasculature of the neokidney in the omentum originated from the host and communicated with the host circulation, suggesting its viability for collecting and filtering the host blood to produce urine.
- Moreover, human eritropoetin (EPO) was also produced by neokidney which able to generate red blood cell synthesis, so the kidney was able to participate in other functions as well.

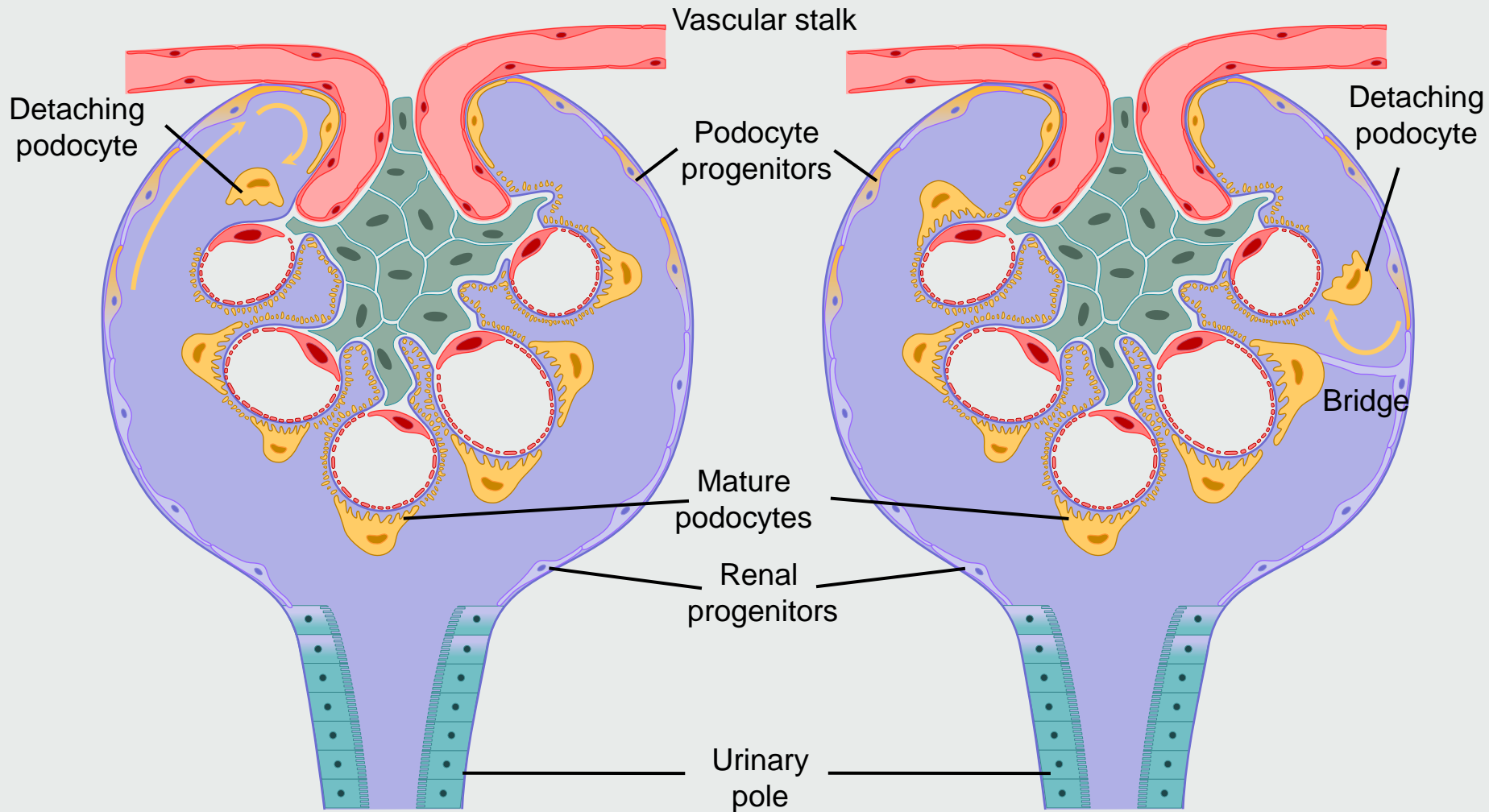
Stem/progenitor cells in the kidney repair

- Different stem cell types exist that possess different degrees of self-renewal as well as pluripotential capability.
- In a normal physiologic state, the kidney possesses very low regenerative capacity compared to other organs, but after insult, for example acute tubular necrosis, tubular epithelial cells can replicate and regenerate damaged tubules.
- Experiments of administration of in-vitro expanded stem cells demonstrate that the mesenchymal stem cells protect and improve the recovery from acute tubular injury induced by ischaemia and chemicals.

Endogenous stem cells

- During embryogenesis most of the renal parenchymal cells are derived from the metanephric mesenchymal cells, which are multipotent and self-renewing.
- In various animal models many progenitors of renal cells have been identified based on their location within the renal compartment including along tubular cells, within Bowman's capsule, the papillary region, and within the cortical interstitium.
- In human kidney, the existence of a population of cells (CD133+/CD24+) located in the urinary pole of the glomerulus. After stimulation they can give rise to tubular epithelial cells as well as podocytes.
- Other cell population (CD133+/CD24+/PDX+) present between the urinary pole and the vascular pole that gives rise to podocytes only.

Renal progenitor cells



Exogenous stem cells

- Stem cells isolated from bone marrow and amniotic fluid, MSC, have been primarily used as exogenous sources for suitable therapy to treat kidney disease.
- Injections of these stem cells seems to cause a functional recovery of the injured kidney.
- It is not clear how these cells are able to act at the site of degenerated tissue.
- The mechanisms could be transdifferentiation and integration into the injured site. In this latter case the efficiency varies between 2%-20%.
- In opposite to this low efficacy the therapy has positive outcomes, maybe due to the autocrine / paracrine mechanisms exerted by secreted cytokines of these injected cells.

Humoral factors released by progenitor cells involved in kidney repair

- Vascular endothelial growth factor (VEGF) attenuates glomerular inflammation and accelerates glomerular capillary repair.
- Hepatocyte growth factor (HGF) is an angiogenic growth factor prevents epithelial cell death and enhances regeneration and remodeling of injured or fibrotic renal tissue.
- Insulin-like growth factor (IGF): enhance glomerular filtration rate and mitogenic for proximal tubule cells
- These effects were individually observed, the joined effects are unknown.

Microvesicles and cell-cell communication

- Release of microvesicles from cells have been observed *in vivo*.
- Microvesicles are derived from the endosomal membrane compartment and after fusion with the plasma membrane are released from the cell surface. The production of microvesicles is initiated by cellular activation by ligands and stress signals.
- Microvesicles can deliver genetic information such as mRNA or even microRNA acting as a vehicle for genetic exchange between cells.
- It is now known that microvesicles may interact with cells through specific receptor–ligand interactions.

Microvesicles and stem cells

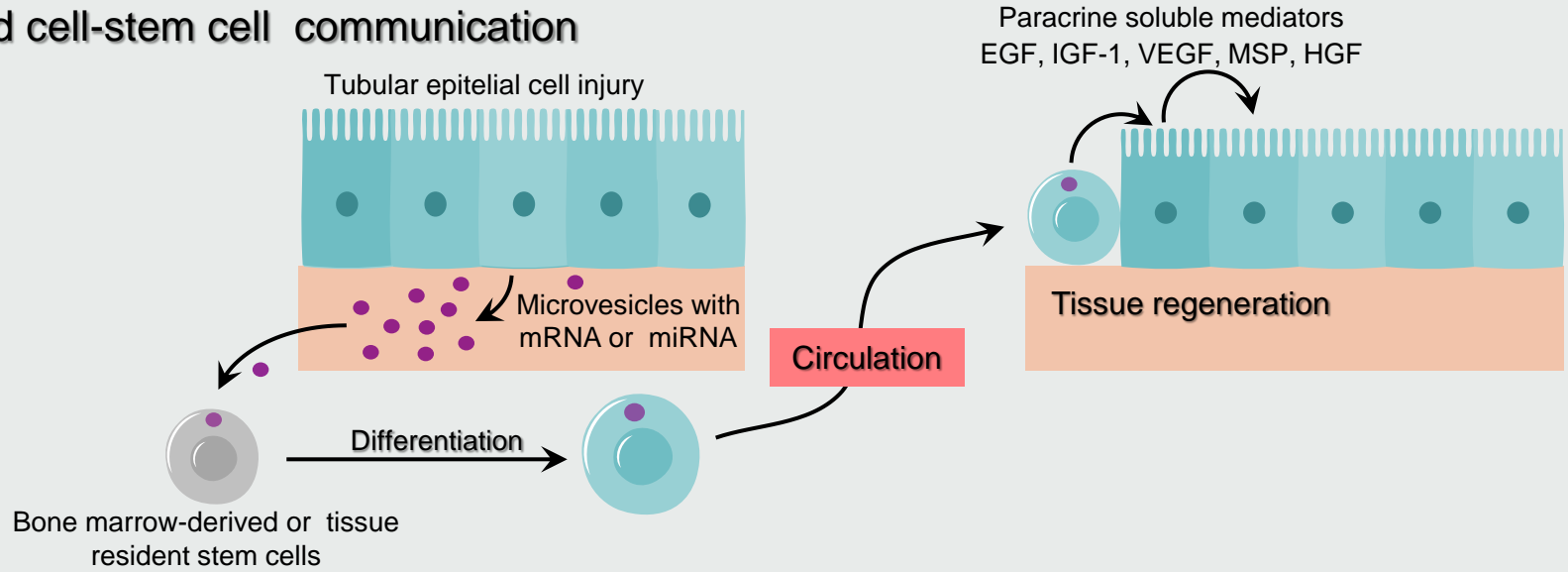
- The phenotype of stem cells is reversibly changing during the cell cycle.
- The same stem cell may show different phenotypes in different functional states, depending on the cell cycle phase.
- This dynamic context is regulated by the microenvironment and in particular the microvesicle-mediated transfer of genetic information between cells.
- Microvesicles generated from endothelial progenitor cells (EPCs) when internalized in normal endothelial cells activate an angiogenic program by a horizontal transfer of mRNA.
- The microvesicles released by ESCs are able to reprogram hematopoietic progenitors by delivery of mRNA and proteins.
- These indicate that stem cells may release biologically active microvesicles not only in their microenvironment but also in the blood as seen after bone marrow mobilization.

Microvesicles role in kidney repair

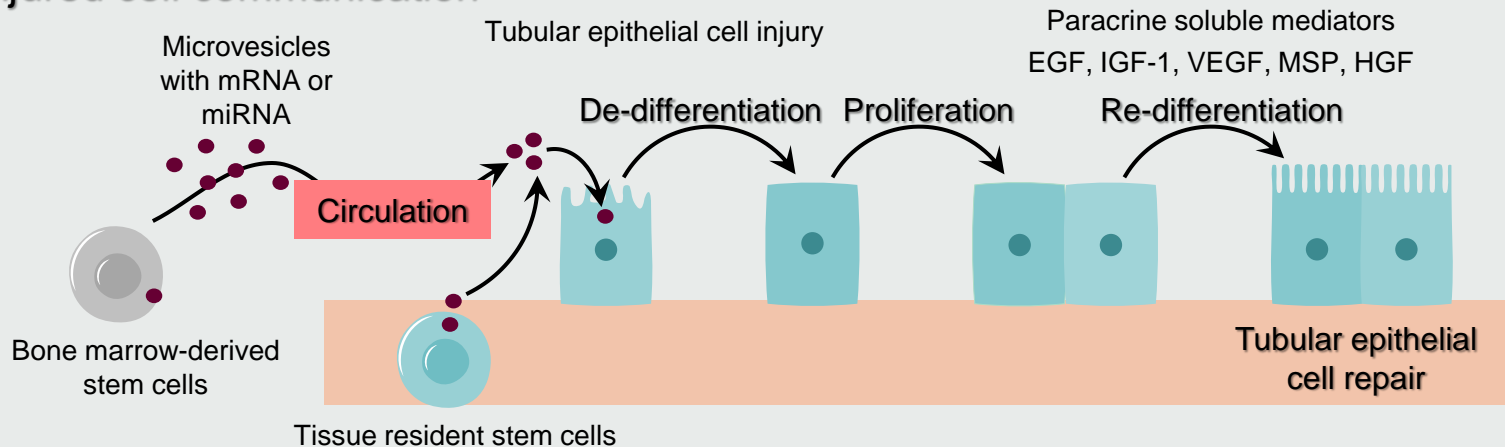
- Human MSCs able to release microvesicles and stimulate *in vitro* proliferation and apoptosis resistance of tubular epithelial cells.
- Moreover, accelerate *in vivo* the functional and morphological recovery of tubular cells in SCID mice with chemical induced AKI.
- RNase treatment of microvesicles abolished both the *in vitro* and the *in vivo* effects of microvesicles, suggesting a mechanism dependent on RNA delivery.
- Microvesicles released from stem cells at the site of tissue injury may induce dedifferentiation of resident cells surviving injury, making them transiently acquire a stem cell-like phenotype with the activation of tissue regenerative programs.

Repair mechanisms of stem cells in kidney regeneration

Injured cell-stem cell communication



Stem cell-injured cell communication



Gene therapy for kidney disease I

Gene therapy for acute kidney failure:

During AKF happen a series of events such as local inflammation, the expression of chemotactic and chemoattractant molecules with higher apoptosis rates and impaired regeneration.

- Blocking NFkB signaling pathways inhibit the cytokine/chemokine (MCP1) activation so abolish the recruitment of inflammatory cells.
- Downregulation of ICAM 1 expression by antisense oligos can decrease the numbers of infiltrating neutrophils.
- Knock down of complement 3 and upregulation of Bcl2 by adenovirus transfection could rescue renal cells from extended apoptosis.
- Hepatocyte growth factor (HGF) therapy can prevent inflammation and organ degeneration.

Gene therapy for kidney disease II

Gene therapy for chronic kidney failure:

- The imbalance between the inflammatory and anti-inflammatory processes is the basics of chronic kidney failure. The major player is the TGF- β cytokine in this injury process promoting fibrosis with collagen deposition.
- Due to its complex signalling and many targets several attempts were made to control TGF- β signaling especially the downstream SMAD (2/3, 4, 7) molecules. Upregulation of SMAD 7 (by this inhibiting SMAD2/3 receptors of TGF- β) could abolish fibrosis and inflammation.
- Moreover, targeting AP-1 or NF κ B gave also successful results.

Ex vivo gene therapy:

- Both regular renal transplantation and stem cell therapy could have benefit from gene therapy controlling immunogenicity of the allogeneic tissues. Some attempts were made using CTLA4-Ig and rIL-10 constructs.

Summary

- Kidney is the one the most highly differentiated organ in our body.
- Kidney failure due to different backgrounds is increasing in human population.
- Considering these facts it is important to enhance the conventional kidney replacements along with novel therapeutical approaches.
- Novel data show that kidney can be regenerated using artificial scaffolds along with somatic cells.
- Moreover, promising applications are emerging using exogenous and endogenous originatad stem cells and various gene therapy for kidney regeneration.