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Regenerative medicine in urology

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ABSTRACT

Repair and reconstruction of damaged tissues and organs has been a major issue in the medical field. Regenerative medicine and tissue engineering, as rapid evolving technologies, may offer alternative treatments and hope for patients with serious defects and end-stage diseases. Most urologic diseases could benefit from the development of regenerative medicine and tissue engineering. This article discusses the role of cells and materials in regenerative medicine, as well as the status of current role of regenerative medicine for the generation of specific urologic organs.

Introduction

Repair and reconstruction of damaged tissues and organs has been a major issue in the medical field. Resection of lesion, Repair with autologous tissue, and Replacement with allografts are the three R's in traditional surgery. However, autologous transplantation is limited by donor site conditions and may cause secondary donor site injury. Allogeneic transplantation is limited by donor organ shortage and may lead to tissue rejection. Therefore, researchers have been studying how to Regenerate damaged tissues and organs with new techniques, which is the fourth "R" in the development of novel treatments. Regenerative medicine is the process of replacing or regenerating of human cells, tissues, or organs to restore or establish normal function.[1] It is a rapidly evolving field, which incorporates tissue engineering, cell biology, biochemistry, material science, and many other disciplines. In order to restore the function of diseased organs, there are generally three different methodical approaches: (1) cell-based therapy, with autologous or allogeneic stem cells obtained from a biopsy and expanded *in vitro* for clinical application; (2) implantation of biological or synthetic materials to assist and guide the repair process; and (3) implantation of matrices that are seeded with cells.

An increasing significance of regenerative medicine and tissue engineering is observed in urology. Almost every urologic tissue and organ is being studied, and some of them have made the translation into clinical application. In this article, we review the general concepts of regenerative medicine in urology, including cells, materials, and major achievements for specific urologic organs.

Cells

Endogenous primary cells are one of the ideal cell sources in regenerative medicine. The use of these cells would prevent tissue rejection and reduce inflammatory issues. Adult urothelial cells have many critical functions, such as barrier against urine toxicity and the expansion ability to adjust to volume changes of the bladder. On the other hand, smooth muscle cells play a key role in urination. Both types of cells have been successfully obtained from bladder biopsy.[2] Scaffolds seeded with these endogenous primary cells can be used to augment or replace the bladder *in vivo*. A major concern is that due to the relatively short lifespan of primary cells *in vitro*, it may be difficult to expand them to a large amount. Furthermore, it may be detrimental to expose a sick patient to an additional procedure, and there may not be enough healthy cells in diseased organs to start the expansion process. However, research showed that smooth muscle cells derived from normal bladders and neurogenic bladders exhibited similar biomarkers. After seeding cells of different origins onto scaffolds, the engineered constructs showed similar contractility both *in vitro* and *in vivo*.[3] Our group has isolated renal cells from normal kidneys and kidneys with chronic kidney disease. Both cell types possessed similar phenotype and proliferation kinetics. Functional tests, including sodium uptake and albumin uptake, demonstrated that cells from both normal and diseased kidneys were comparable. Therefore, even cells from diseased organs may be candidates for cell therapies in the future. Besides, for many cell types, detailed protocols have now been developed to expand specific cells into large quantities *in vitro*.[4] The application of techniques, such as mesh grafting, allows us to create constructs 3–6 times larger with the same amount of cells.

Adult stem cells (ASCs), also known as resident stem cells, are within tissues or organs of an adult. Maintenance of tissue

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homeostasis and regeneration after minor injury are the result of proliferation and differentiation of these ASCs.[5] The earliest application of ASCs was the allogeneic bone marrow stem cell transplantation.[6] Until now, ASCs have been successfully isolated from nearly every tissue inside the human body.[7] The advantages of ASCs are as follows: these cells can be autologous; the use of ASCs does not have ethical obstacles; depending on the locations, some ASCs are relatively easy to access and isolate; and they are not likely to form tumors after transplantation. Bone marrow-derived stem cells (BMSCs) are able to generate cartilage in a number of experiments and some clinical trials. Encouraging clinical results have been reported by Wong et al.[8] Patients with unicompartmental osteoarthritic knees and genu varum underwent microfracture and medial opening-wedge high tibial osteotomy. Then, the patients received intra-articular injection of BMSCs or served as control. After 2 years of follow-up, patients injected with BMSCs showed better cartilage improvement compared with control group. A major limitation is that the number of ASCs is low in each tissue and that it may be difficult to greatly expand ASCs *in vitro* without differentiation. Therefore, to culture enough cells for tissue engineering and clinical application is challenging.

Embryonic stem cells (ESCs) are isolated from the inner cell mass of human blastocyst.[9] There are two important features possessed by ESCs: the unlimited ability to self-renew through mitosis and the potential to differentiate into a large range of specialized cells from all the three germ layers, ectoderm, mesoderm, and endoderm.[10] Because of these two essential features of ESCs, they have been expanded and induced to urothelial cells and smooth muscle cells in large quantities.[11] The differentiated cells can be an alternative cell source in bladder regeneration. Of course, there are several problems to be solved before the application of ESCs in clinic. In the first reports of ESCs, mouse embryonic fibroblast feeder layers are applied to provide both growth factors and proper support. Nonetheless, for clinical applications, derivation, maintenance, and differentiation procedures should be accomplished under xeno-free conditions using good manufacturing practice (GMP) systems.[12] Currently, human feeder cells from uterine endometrium, bone marrow, and many other tissues have replaced mouse embryonic fibroblasts, and successful derivations of ESCs without feeder cells are also now common.[13–15] These approaches may eliminate the risks of xeno-contamination. The use of ESCs is associated with ethical and political issues due to the source of these cells. Tumorigenicity is another limitation of ESCs. Various strategies have been tested, including ablation of residual pluripotent stem cells or tumor progression-specific genes and early tumor detection and elimination in patients.[16] There are on-going clinical trials with ESCs for patients with macular degeneration.[17]

In order to obtain pluripotent cells without destructing embryos, researchers have focused on pluripotency-inducing factors. In 2006, Takahashi and Yamanaka[18] introduced four transcription factors (OCT3/4, SOX2, c-MYC, and KLF4) into murine fibroblasts and reversed them into pluripotent cells. These reprogrammed cells were called induced pluripotent stem cells (iPSCs), and they possessed similar properties as ESCs regarding morphology, phenotypic markers, proliferation properties, differentiation *in vitro*, and teratoma formation when injected into immunocompromised mice. Even though the use of iPSCs does not raise ethical issues, there are other questions to be answered. C-MYC, a proto-oncogene, being one of the transcription factors has lead to much concern. Yu et al.[19] successfully generated iPSCs with OCT4, SOX2, NANOG, and LIN28. Other combinations of transcription factors without c-MYC have also been reported. The removal of c-MYC was found to be critical to prevent the cells from becoming potentially tumorigenic.[20] These transcription factors were originally delivered by retro- or lentiviral constructs. Such multiple integration sites may cause insertional mutagenesis and endogenous oncogene activation. A possible alternative would be non-viral-based methods and adenovirus-based transient method without genomic integration.[21–24] Although iPSCs share the problem of tumorigenicity, they have several advantages over ESCs. Compared with isolating cells from inner cell mass of blastocysts, it is less complicated to generate iPSCs by following standard protocols. iPSCs are also autologous cells, minimizing the risks of tissue rejection by host immune system.

Amniotic fluid-derived stem cells (AFSCs) are an important representative of perinatal stem cells. Amniotic fluid is in direct contact with a variety of embryonic components, and protects and aids the development of the fetus *in utero*. The best-characterized AFSCs population was first described in 2007.[25] The cells can be isolated by positive selection for the surface marker c-kit through cell sorting. Undifferentiated AFSCs have the ability to proliferate without feeder cells to a great extent. AFSCs can be maintained in culture for over 250 doublings, and following extensive expansion, the cells retained long telomeres and a normal karyotype. These stem cells accounted for approximately 1% of the cellular components of amniotic fluid. AFSCs expressed markers of both ESCs and mesenchymal stem cells (MSCs). They express SSEA-4 and OCT4, which were common markers in ESCs. Meanwhile, they express mesenchymal stem cell markers, such as CD29, CD44, CD73, CD90, and CD105.[25] The presence of these markers suggests that AFSCs are at an intermediate stage between ESCs and adult stem cell phenotypes. Unlike ESCs and iPSCs, AFSCs do not induce teratoma formation when injected *in vivo*, and they do not raise critical ethical questions. AFSCs, together with other perinatal stem cells such as umbilical cord placenta and amnion membrane-derived stem cells, may be preserved as a lifelong autologous cell source in case of a life-threatening disease.[5,26] In the field of pediatrics especially, when prenatal defects are detected, amniotic fluid samples can be obtained by amniocentesis. Autologous AFSCs can be isolated, expanded *in vitro*, and differentiated for tissue engineering and reconstruction.

Biomaterials

Traditionally, biomaterials have been used as an extracellular matrix (ECM) to support the cells against *in vivo* forces and to provide physical adhesiveness. They can also be loaded with bioactive factors, such as growth factors and cytokines, to further support the cells. However, recent years have witnessed the growing understanding that ECM should also play an important role in regulating cell functions and behavior, including gene expression, proliferation, migration, differentiation, and survival. For example, matrix materials with fibroblasts are often used in research for the treatment of skin wounds. Fibroblasts expressing migration-stimulating factor (MSF), an angiogenic cytokine, have the ability to migrate to larger extent, which is critical in wound healing.[27] Fibroblasts derived from normal adult skin can be induced to express MSF by a transient treatment, TGF-β1. However, the induction can only be achieved when cells are seeded onto a "wound" matrix, such as denatured type I collagen.[27] Another example would be muscle stem cells (MuSCs). Vigorous regenerative capacity of MuSCs has been observed *in vivo*. Yet, after being cultured *in vitro*, this capacity is rapidly lost. Gilbert et al.[28] used soft hydrogel substrates to simulate key niche features. MuSCs cultured on this substrate, which mimicked the elasticity of muscle, were able to self-renew *in vitro* and contributed greatly to muscle regeneration after being translated into mice. When engineering sphincter muscles of the urinary tract, similar strategies may be beneficial.

There are generally three classes of biomaterials utilized in the field of regenerative medicine: naturally derived materials,