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Review Article

# Tissue Engineering in Vesical Reconstruction

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## ABSTRACT

**Objectives:** This review summarizes the basic principles of tissue engineering (TE) and describes the possible future clinical application in bladder reconstruction.
**Material and Methods:** This review is based on an electronic search of the PubMed database and recently published presentations between November 2008 and May 2009 about basic research on TE and vesical reconstruction.
**Results**: Few articles (about 30) described bladder reconstruction utilizing TE approaches, most being reviews, with 8 experimental animals studies, and only one study in human subjects.
**Conclusion:** Despite the fact that TE is a recently developed field and remains largely experimental, it promises to influence urological treatment in the near future. One can predict that some form of engineered urothelial tissue will enter the clinical domain within the next 5 to 10 years.

**Key Words :** Bladder, reconstructive urology, stem cells, tissue engineering

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## INTRODUCTION

Tissue engineering (TE) is one of the major components of regenerative medicine. Scientists in that field follow the principles of cell transplantation, materials science and engineering to develop biologic substitutes that can restore and maintain normal function in a diseased organ[1]. Although the goals of TE are ambitious and have not yet been attained, significant milestones have been achieved and future possibilities are substantial. Engineered organs could sidestep many of the problems associated with donor organs, and at lower cost. Other equally promising applications, include conditions associated with tissue loss, such as strictures of the ureter and urethra, hypospadias, exstrophy-epispadias complex, bladder dysfunction in patients with myelomeningocele or traumatic spinal cord injury, and cystectomy for invasive bladder cancer[2].

## MATERIAL AND METHODS

This review is based on a search of the PubMed database and recently published presentations at international Urology meetings. The literature analysis was performed between November 2008 and May 2009, and was accompanied by comparative discussions of the current primary trends in TE basic research, experimental animal studies and clinical trials in vesical reconstruction.

## RESULTS

Despite a marked increase in the number of articles about TE and stem cell research in the past few years, few papers describe bladder reconstruction utilizing TE approaches. The purpose of this article is to review recent developments in augmentation or substitution of the bladder. The described techniques have been successfully employed in experimental animal studies or in vitro systems, but only one study by Atala and colleagues (2006) has described augmentation cystoplasty in human subjects utilizing TE approaches.

Several varieties of TE techniques are currently under investigation, yet so far no single approach has been clearly superior based on significant long-term studies. The main goals of all techniques were to determine the opti-

mum scaffold that can be seeded by cells, and the best source of stem cells.

## I- BASIC PRINCIPLES OF TISSUE ENGINEERING

TE strategies generally fall into two categories: the use of acellular matrices (tissue regeneration) or the use of cellular matrices.

A- The use of acellular matrices depends on the body's natural ability to generate new tissue growth. Acellular tissue matrices are usually prepared from biomaterials to form artificial scaffolds. These matrices tend to slowly degrade on implantation and are generally replaced by extracellular matrix (ECM) proteins secreted by ingrowing cells[3].

B- The use of matrices with cells depends (the subject of this sentence is "use", not "cells") upon an autologous sample of urothelial and smooth muscle cells from the host tissue, obtained by small biopsies. These cells are either implanted directly into the host, or reimplanted into the host after expansion in a cell culture[4].

Autologous cells are the preferred type, as it does not induce host immune response, but there is a theoretical problem in using autologous cells from diseased organs. Investigators reported a significant difference in the functional characteristics of cells obtained from normal and diseased organs, and the clinical impact of this difference should be explored[5].

### 1- The role of cell culture and cell expansion

Recent development of cell culture techniques allows (the subject of the sentence is "development", not "techniques") the expansion of a urothelial strain from a single specimen that initially covered a surface area of $1cm^2$, to one covering a surface area of 4202 $m^2$ within 8 weeks (the equivalent of one football field). These studies indicated that it should be possible to collect autologous bladder cells from human patients, expand them in culture and return them to the donor in sufficient quantities for reconstructive purposes[6].

The success of cell cultures depends on utilizing recent techniques, such as fluorescence-activated cell sorting, immunomagnetic bead sorting, and magnetic-activated cell sorting. These recent techniques enabled the selection of specific cell types, and the targeting of stem cell differentiation to the required cell type[7].

### 2- The role of biomaterials

Biomaterials are essential for acellular or cell-based TE techniques. Biomaterials replicate the biologic and mechanical function of the native ECM proteins; in addition, they provide a three-dimensional space to form new tissues. Moreover, they can allow delivery of growing cells to the desired sites, and loading of bioactive factors that regulate cell function and behaviour.The ideal biomaterial should be biodegradable within a certain time, bioresorbable to support replacement of normal tissue without inflammation, and biocompatible to avoid foreign-body response[8].

Generally, three classes of biomaterials are used: (1) naturally derived materials that include collagen, the most abundant structural protein in the body,[9] and alginate, a polysaccharide isolated from sea weed;[10] (2) acellular tissue matrices (decellularized bladder submucosa or small intestinal submucosa) which are collagen-rich matrices prepared by removing cellular components from small intestine mucosa or bladder mucosa;[11] (3) synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and polylactic-co-glycolic acid (PLGA), which are polyesters of naturally occurring á-hydroxy acids[11].

### 3- The role of stem cells in TE

Most current strategies for TE depend upon a sample of autologous cells from the diseased organ of the host. However, in cases

of end-stage organ failure, a tissue biopsy may not yield enough normal cells, or autologous cells cannot be expanded. In these situations alternative sources of cells can be derived from pluripotent stem cells by therapeutic cloning.12 Stem cells can be derived from discarded human embryos (human embryonic stem cells), from fetal tissue, from adult tissue (skin, fat, muscle, bone marrow, testes) and from amniotic fluid[13].

There are two types of nuclear cloning: reproductive cloning and therapeutic cloning. Reproductive cloning is used to generate an embryo that has identical genetic material as its cell source. This embryo can then be implanted into the uterus of a female to give rise to an infant that is a clone of the donor individual. In 1997 a sheep named Dolly was the first mammal to be produced from adult somatic cells utilizing nuclear transfer techniques. In therapeutic cloning a donor nucleus is transferred into an enucleated oocyte to produce an early stage embryo, which is explanted into a tissue culture to provide a limitless source of human embroyonic stem cells whose genetic material is identical to that of its source. These autologous stem cells have the potential to proliferate into an undifferentiated but pluripotent state (self-renewal) and has the ability to differentiate into many specialized cells of any type in the adult body. Therefore, it is considered the basis for cell transplantation and organ replacement applications, without inducing a host immune response or the need for a donated organ; it is likely to replace the use of autologous organ-specific cells in the future[14].

The production of stem cells by therapeutic cloning has many limitations. It includes ethical issues, low efficiency of the cloning process and consumption of a large number of oocytes. Other problems include the scarcity of donated oocytes, possible congenital abnormalities, and the need to create scores of embryos to establish a single line of stem cells[15]. There are several trials to improve cloning efficiency by proper epigenetic reprogramming of the somatic cell to an embryonic state[16].

## II-TISSUE-ENGINEERING IN VESICAL RECONSTRUCTION

### Introduction

The stimulus for TE research in vesical reconstruction arises from the failure to reconstruct a bladder lined by urothelium. The benefits of enterocystoplasty carry a price, in the form of significant, well-documented complications that include mucus production, stone formation, bacteriuria, metabolic disturbances, intestinal obstruction, and even malignancy[17].

Several alternatives have been explored to reconstruct a bladder lined by urothelium. These include the use of native urothelium derived either from a grossly dilated ureter (ureterocystoplasty) or from the bladder wall itself after excising the overlying detrusor muscle (auto-augmentation). Ureterocystoplasty is an attractive and clinically proven concept, but one which is effectively confined to a small minority of patients with gross ureteric dilatation. The long-term functional outcome of bladder auto-augmentation has been disappointing[18]. Recent advances in the field of nanotechnology and stem cell biology provide alternative treatment by TE and neuromodulation[19].

The strategy of TE to reconstruct a bladder lined by urothelium can be grouped into two broad approaches: (1) in vivo tissue regeneration and (2) in vitro TE utilizing two techniques, the cell-seeded, and non-seeded technology.

Cell-seeded technology uses scaffolds that are seeded in vitro with primary cultured cells obtained from a bladder biopsy. This composite graft is then implanted back in the host. The non-seeded technology uses cell-free biodegradable scaffolds to allow the natural process of regeneration to occur in vivo[20].

It is unclear which approach is preferable. An experimental animal study comparing seeded and non-seeded scaffolds to reconstruct the bladder in a canine model of subtotal cystectomy recommended that cells are

necessary to achieve improved bladder tissue function, especially when a large amount of bladder tissue is required[21].

**Tissue engineering approaches**

**1- In vivo tissue regeneration using acellular matrices**

Taking advantage of the ability of the bladder to undergo rapid regeneration and repair after acute injury, a biomaterial implanted at the time of surgery becomes cellularized and eventually is assimilated into the tissues of the host bladder. Of all the synthetic and natural materials used to date, decellularized xenogeneic or allogeneic matrices prepared from the submucosa of small intestine and bladder have shown greatest promise. Experimental animal studies in rodent, canine and porcine models reported good tissue morphology and functional regeneration, but only to replace 30% to 40% of the required amount of bladder tissue[22].

Experimental studies with longer followup reported graft contraction and fibrosis leading to diminished bladder capacity. Due to the obvious advantage as an "off-the-shelf" solution for reconstructive bladder surgery, the authors preferred matrices implanted with cells to achieve the required amount of engineered bladder tissue with good function[23].

**2- In vitro tissue engineering**

**A- Composite enterocystoplasty**

The potential complications of conventional enterocystoplasty are largely due to the unsuitability of the intestinal epithelium rather than the smooth muscle component of the bowel wall. For this reason, cell-culture techniques are employed to generate adequate amounts of autologous urothelium for use in combination with de-epithelialized bowel segments. The observation of areas with incomplete urothelium covering, and others with re-growth of inappropriate host epithelium, prevents (subject of the sentence is "observation") composite enterocystoplasty from being realized as a clinical approach for bladder reconstruction. Nevertheless, it is an attractive strategy, as it requires only one component of the neobladder to be engineered[24].

**B- Tissue engineered autologus neobladder in experimental animals**

Bioengineering has allowed creation of functional neo-bladder tissues in several animal models. Autologous cells obtained by biopsy from the host were used to avoid rejection.The cells are dissociated and expanded in vitro, attached to a biodegradable matrix and re-implanted into the same host. After implantation, histological evidence showed that the engineered bladders continued to develop until they appeared normal anatomically and functionally[25].

The local and systemic effects of autologous neobladders engineered in canine models have been studied for 6 months postoperatively. The capacity and compliance of the engineered neobladder increased over time to normal levels. In addition, histological and immunohistochemical analyses showed that the engineered neobladder had adequate structural architecture, similar to that of native bladder. All other parameters including blood chemistry, urodynamics, cystography and urine analysis were normal[26]. Current research suggests that bladders constructed in vitro may derive benefit from regular mechanical stimulation by a bladder tissue bioreactor. Such stimulation appears to induce favorable cellular changes essential for urinary bladder physiology[27].

**C-Tissue engineered autologus neobladder in humans**

Atala and colleages (2006), after 17 years of basic research, conducted a clinical trial in seven patients with myelomeningocele, aged 4-19 years, with high-pressure or poorly compliant bladders and failure of medical treatment. A bladder biopsy was obtained from each patient. Urothelial and muscle cells were grown in culture, and seeded on a biodegradable bladder-shaped scaffold made

of a composite of collagen and polyglycolic acid for 7 weeks. The autologous engineered bladder constructs were implanted either with or without an omental wrap. The mean follow-up extended for 46 months. Serial urodynamics, cystography, ultrasound, bladder biopsy and serum biochemistry were done. Post-operative cystography and urodynamic studies showed improvement, especially in patients whose engineered bladder was covered with an omental wrap. Bowel function returned promptly after surgery with no metabolic consequences noted. Urinary calculi did not form, furthermore mucus production was normal, and renal function was preserved. They concluded that engineered bladder tissues, created with autologous cells seeded on collagen-polyglycolic acid scaffolds, and wrapped in omentum after implantation, could be used in patients who require cystoplasty[28].

## DISCUSSION

Dramatic advances in the fields of biochemistry, cell and molecular biology, genetics, biomedical engineering and materials science have given rise to the remarkable new cross-disciplinary field of TE. Several technologies come together in TE that creates many controversies and difficulties to master all technological aspects.

TE holds the promise for a tremendous impact on reconstructive urology. However, research must be intensified for the full potential clinical benefits to be made widely available. Engineered organs could avoid many of the hazards and problems associated with donor organs, and at lower cost. One of the most promising technologies for making artificial organs derives from work by Atala in the Wake Forest Institute for Regenerative Medicine, Winston-Salem, USA[28]. Atala has pioneered the field both as practitioner and as theorist in creating a functioning neobladder using a patient's own cells.The engineered bladder was tailored to the individual and organs cannot yet be made for "off-the-shelf" distribution. Recently a phase 2 clinical trial of the Tengion Neo-Bladder Augment™ for children with neurogenic bladder due to spina bifida was filed and accepted by the Food and Drug Administration. A clinical trial in patients with bladder cancer requiring total cystectomy will be initiated in the first half of 2010.

In addition to resolving all specific basic research aspects of TE, legal discussions must support the movement of TE from theory to clinical application. Also, several areas of potential concern should be highlighted, including the possibility of introducing pathogenic agents, neoplastic transformation or congenital malformations resulting from TE[29].

In conclusions; Despite the fact that TE is a recently developed and largely experimental field, it promises to influence urological treatment in the near future. Autologous neobladder cystoplasty had been applied successfully in humans, and it seems reasonable to predict that some form of urothelial TE will enter the clinical domain within the next 5 to 10 years.

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