Stratégies de l'ingénierie tissulaire

Tissue Engineering Strategies

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Mots clés

- Médecine régénérative
- Ingénierie tissulaire
- Substituts biologiques
- Cellules souches

Résumé

Les applications de la technologie en médecine régénérative peuvent offrir de nouvelles thérapeutiques aux patients porteurs de lésions, de défaillances terminales d'organes ou d'autres problèmes cliniques.
Actuellement, les patients souffrant d'organes malades ou lésés peuvent être traités par transplantation d'organes. Cependant il y a une grande pénurie de donneurs qui va en s'aggravant chaque année, car la population vieillit et les nouveaux cas de défaillance d'organes augmentent. Les scientifiques dans le domaine de la médecine régénérative et l'ingénierie tissulaire appliquent maintenant les principes de transplantation cellulaire, de la science des matériaux et de la bio-ingénierie pour construire des substituts biologiques qui restaureront et maintiendront une fonction normale à des tissus malades ou atteints. Le domaine de la cellule souche est en train d'avancer rapidement, ouvrant de nouvelles avenues pour ce type de thérapeutique.
Par exemple, le clonage thérapeutique et la reprogrammation cellulaire peuvent un jour fournir une source potentielle illimitée aux cellules pour des applications en ingénierie tissulaire. Bien que les cellules souches soient encore au stade de la recherche, certaines thérapeutiques issues de l'ingénierie tissulaire sont déjà entrées avec succès dans un cadre clinique, annonçant la promesse que la médecine régénérative tient pour l'avenir.

Keywords

- Regenerative medicine
- Tissue engineering
- Biological substitutes
- Stem cells

Abstract

Applications of regenerative medicine technology may offer novel therapies for patients with injuries, end-stage organ failure, or other clinical problems. Currently, patients suffering from diseased and injured organs can be treated with transplanted organs. However, there is a severe shortage of donor organs that is worsening yearly as the population ages and new cases of organ failure increase. Scientists in the field of regenerative medicine and tissue engineering are now applying the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. The stem cell field is also advancing rapidly, opening new avenues for this type of therapy. For example, therapeutic cloning and cellular reprogramming may one day provide a potentially limitless source of cells for tissue engineering applications. While stem cells are still in the research phase, some therapies arising from tissue engineering endeavors have already entered the clinical setting successfully, indicating the promise regenerative medicine holds for the future.

Patients suffering from diseased and injured organs may be treated with transplanted organs. The use of one body part for another or the exchange of parts from one person to another was mentioned in the medical literature even in antiquity. Charles Lindbergh, the first pilot to successfully fly across the Atlantic in the 1920s, joined forces with Alexis Carrel, a Nobel Prize Winner in the field of Medicine, to investigate the potential of keeping organs alive ex-vivo long term (1).
The kidney was the first entire organ to be replaced in a human, in 1955 (2). However, this transplant was performed between identical twins, and thus it did not address the immune response to transplanted organs. In the early 1960s, Murray, who later received the Nobel prize for his work, performed a nonrelated kidney transplantation from a non-genetically identical patient into another. This transplant, which overcame the immunologic barrier, marked a new era in medical therapy and opened the door for use of transplantation as a means of therapy for different organ systems. However, lack of good immune-suppression, the inability to monitor and control rejection, and the worsening donor shortage spurred physicians and scientists to look for other alternatives.
Synthetic materials were introduced to replace or rebuild diseased tissues or parts in the human body. The advent of new manmade materials, such as tetrafluoroethylene (Teflon) and silicone, opened a new field which included a wide array of devices that could be applied for human use. However, although these devices could provide for structural replacement, the functional component of the original tissue was not achieved. Meanwhile, new techniques for cell harvesting,

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culture, and expansion were developed. Studies of the extracellular matrix and its interaction with cells, and with growth factors and their ligands, led the way to a further understanding of cell and tissue growth and differentiation. The concept of cell transplantation took hold in the research arena, and culminated with the first human bone marrow cell transplant in the 1970s. At this time, researchers began to combine the devices and materials with cell biology concepts, creating a new field called *tissue engineering.* As more scientists from different fields came together with the common goal of tissue replacement, the field of tissue engineering became more formally established.

The fields of cell transplantation and tissue engineering have also been combined with stem cell biology in the past three decades. These areas all had one unifying concept : the regeneration of living tissues and organs. Thus in 1999, William Haseltine, then the Scientific Founder and Chief Executive Officer of Human Genome Sciences, coined the term "regenerative medicine", in effect bringing all these areas under one defining field (3). While organ transplantation remains a mainstay of treatment for patients with severely compromised organ function, the number of patients in need of treatment far exceeds the organ supply, and this shortfall is expected to worsen as the global population ages. However, recent advances in regenerative medicine suggest that it can provide new alternatives to donor organs. In the last two decades, scientists have attempted to grow native and stem cells, engineer tissues, and design treatment modalities using regenerative medicine techniques for virtually every tissue of the human body. This article reviews some of the progress that has been achieved in the field of tissue engineering.

The basics of tissue engineering

Tissue engineering employs aspects of cell biology and transplantation, materials science, and biomedical engineering to develop biological substitutes that can restore and maintain the normal function of damaged tissues and organs. These techniques can include injection of functional cells into a nonfunctional site to stimulate regeneration and/or the use of biocompatible materials to create new tissues and organs; thus, the two most basic components of tissue engineering strategies are cells and biomaterials. The introduction of cells is designed to stimulate regeneration, promote vascularization, and/or supplement the production of hormones and growth factors. Biomaterials, which include both natural and synthetic matrices (commonly called "scaffolds") are important tools in regenerative medicine. In addition to guiding the direction of new tissue growth and providing the proper spatial environment to restore tissue structure and function, biomaterials may introduce bioactive factors (4, 5) or may attract cells and growth factors from the body following implantation (6, 7). If a biomaterial is implanted without cells, the objective is to encourage the body's natural ability to repair itself.

Cell Types Used in Tissue Engineering

Native Targeted Progenitor Cells

One of the limitations of applying cell-based techniques to organ replacement has been the inherent difficulty of growing specific cell types in large quantities. Even when some organs, such as the liver, have a high regenerative capacity *in vivo*, cell growth and expansion *in vitro* may be difficult. By noting the location of progenitor cells within a tissue, as well as by exploring the conditions that promote differentiation and/or self-renewal, it has been possible to overcome some of the obstacles that previously limited cell expansion *in vitro*. One example of this is the culture of urothelial cells. Urothelial cells could be grown in the laboratory setting in the past, but only with limited success. It was believed that urothelial cells had a natural senescence program that was hard to overcome. However, over the last two decades, several protocols have been developed that have improved urothelial growth and expansion (8-11). A system of urothelial cell harvesting was developed that does not use any enzymes or serum and has a large expansion potential. Using these methods of cell culture, it is possible to expand a few urothelial cells from a single tissue specimen that initially covers a surface area of 1 cm^2 to a surface area of 4202 m^2 (the equivalent area of one football field) within 8 weeks (8).

An additional advantage in using native cells is that they can be obtained from the specific organ to be regenerated, expanded, and used in the same patient without rejection, in an autologous manner (8, 12-28). Bladder, ureter, and renal pelvic cells can all be harvested, cultured, and expanded in a similar fashion, and thus, normal human bladder epithelial and muscle cells can be efficiently harvested from surgical material, extensively expanded in culture, and their differentiation characteristics, growth requirements, and other biologic properties can be studied (8, 10, 11, 21, 22, 29-36). These culture techniques make the use of autologous cells possible for clinical application.

Stem cells and other pluripotent cell types

However, even with the advances in cell culture techniques described above, not all human cells can be grown or expanded in vitro. Liver, nerve, and pancreas are examples of human tissues where the technology is not yet advanced to the point where these cells can be grown and expanded. In this case, it is envisioned that in the near future, stem cells and other pluripotent cell types will be used instead of native cells.

Human embryonic stem cells exhibit two remarkable properties: the ability to proliferate in an undifferentiated but pluripotent state (self-renewal), and the ability to differentiate into many specialized cell types (37). This makes them an attractive resource for regenerative medicine techniques. They can be isolated by aspirating the inner cell mass from the embryo during the blastocyst stage (5 days post-fertilization), and they have been shown to differentiate into cells from all three embryonic germ layers *in vitro*. Skin and neurons have been formed, indicating ectodermal differentiation (38-41). Blood, cardiac cells, cartilage, endothelial cells, and muscle have been formed, indicating mesodermal differentiation (42-44). Pancreatic cells have also been formed, indicating endodermal differentiation (45). However, the ethical, religious, and political controversies surrounding human embryonic stem cells limit their use. New stem cell technologies (such as cloning and reprogramming) promise to overcome this limitation.

Therapeutic cloning (somatic cell nuclear transfer, or SCNT) is used to generate early stage embryos from an oocyte and the nucleus of a somatic cell. These are explanted in culture to produce embryonic stem cell lines whose genetic material is identical to that of its source. These autologous stem cells have the potential to become almost any type of cell in the adult body, and thus would be useful in tissue and organ replacement applications (46). Figure 1 shows the strategy of combining therapeutic cloning with tissue engineering to develop tissues and organs. However, while promising, somatic cell nuclear transfer technology has a number of limitations that require further improvements before therapeutic cloning can be applied widely in replacement therapy.

Recently, exciting reports of the successful transformation of adult cells into pluripotent stem cells through a type of genetic "reprogramming" have been published. Reprogramming is a technique that involves de-differentiation of adult somatic cells to produce patient-specific pluripotent stem cells, without the use of embryos. Cells generated by reprogramming would be genetically identical to the somatic cells (and thus, the patient who donated these cells) and would not be

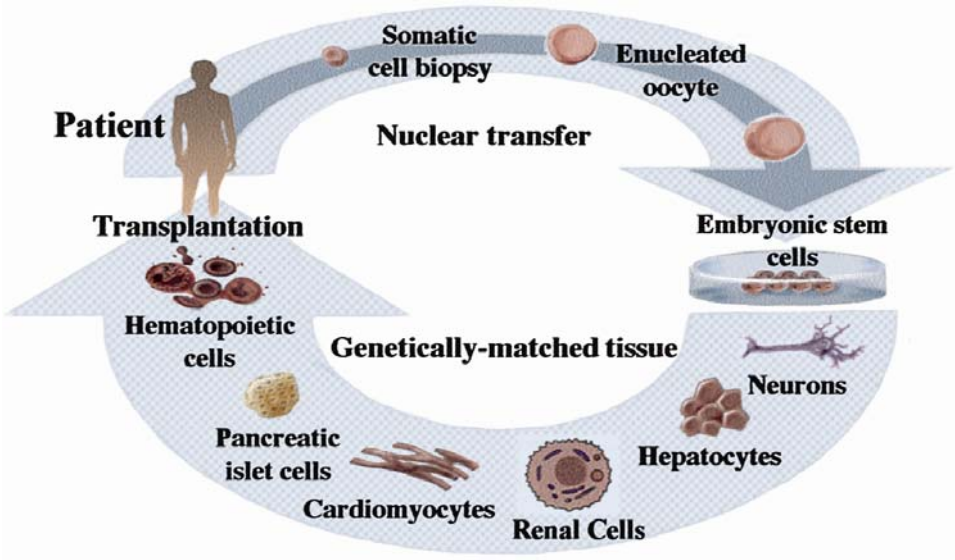

Figure 1 - Strategy for therapeutic cloning and tissue engineering.

rejected. Yamanaka was the first to discover that mouse fibroblasts could be reprogrammed into an "induced pluripotent state (iPS)" (47). The resultant iPS cells possessed the immortal growth characteristics of self-renewing ES cells, expressed genes specific for ES cells, and generated embryoid bodies *in vitro* and teratomas *in vivo*. It has recently been shown that reprogramming of human cells is possible (48, 49), suggesting that this technique may be useful in the future for regenerative medicine strategies.

Finally, other sources of stem cells are the amniotic fluid and placenta. Amniotic fluid and the placenta are known to contain multiple partially differentiated cell types derived from the developing fetus. We isolated stem cell populations from these sources, called amniotic fluid and placental stem cells (AFPSC) that express embryonic and adult stem cell markers (50). AFS cells are broadly multipotent and have been shown to differentiate into cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. In this respect, they meet a commonly accepted criterion for pluripotent stem cells, without implying that they can generate every adult tissue. These cells can be obtained either from amniocentesis or chorionic villous sampling in the developing fetus, or from the placenta at the time of birth, and could be preserved for self use, and used without rejection, or they could be banked.

Biomaterials

For cell-based tissue engineering, cells are seeded onto a scaffold created from an appropriate biomaterial. In tissue engineering, biomaterials replicate the biologic and mechanical function of the native extracellular matrix (ECM) found in tissues in the body by serving as an artificial ECM. Biomaterials provide a three-dimensional space for the cells to form into new tissues with appropriate structure and function, and also can allow for the delivery of cells and appropriate bioactive factors (e.g. cell adhesion peptides, growth factors), to desired sites in the body (51). As the majority of mammalian cell types are anchorage-dependent and will die if no cell-adhesion substrate is available, biomaterials also provide a cell-adhesion substrate. Biomaterials can also provide mechanical support against *in vivo* forces such that the predefined three-dimensional structure is maintained during tissue development.

Generally, three classes of biomaterials have been used for engineering tissues and organs: naturally derived materials, such as collagen and alginate (52-57); acellular tissue matrices, such as bladder submucosa and small-intestinal submucosa (SIS); and synthetic polymers, such as polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactic-co-glycolic acid) (PLGA). Naturally derived materials and acellular tissue matrices have the potential advantage of biologic recognition, but synthetic polymers can be produced reproducibly on a large scale with controlled properties of strength, degradation rate, and microstructure.

Most recently, tissue engineering studies have focused on the use of acellular matrices and synthetic materials. Acellular tissue matrices are collagen-rich matrices prepared by removing cellular components from tissues. The matrices are often prepared by mechanical and chemical manipulation of a segment of tissue (24, 58-60). The matrices slowly degrade after implantation and are replaced and remodeled by ECM proteins synthesized and secreted by transplanted or ingrowing cells. Acellular tissue matrices have been proved to support cell ingrowth and regeneration of genitourinary tissues, including urethra and bladder, with no evidence of immunogenic rejection (60, 61).

On the other hand, polyesters of naturally occurring ¤-hydroxy acids, including PGA, PLA, and PLGA, are widely used in regenerative medicine. These polymers have gained FDA approval for human use in a variety of applications, including sutures (62). The degradation products of PGA, PLA, and PLGA are nontoxic, natural metabolites that are eventually eliminated from the body in the form of carbon dioxide and water (62). Because these polymers are thermoplastics, they can easily be formed into a three-dimensional scaffold with a desired microstructure, gross shape, and dimension by various techniques (63-65). More recently, electrospinning has been used to quickly create highly porous scaffolds in various conformations (66-69). However, one drawback of the synthetic polymers is lack of biologic recognition, although a number of groups are attempting to design synthetic scaffolds which incorporate proteins or other molecules to assist in recognition (70-72).

Tissue engineering of specific structures

Investigators around the world, including our laboratory, have been working towards the development of several cell types and tissues and organs for clinical application.

Urethra

Various strategies have been proposed over the years for the regeneration of urethral tissue. Woven meshes of PGA without cells (73, 74 or with cells {Atala, 1992 #208) were used to regenerate urethras in various animal models. Naturally derived collagen-based materials such bladder-derived acellular submucosa (60), and an acellular urethral submucosa (75), have also been tried experimetnally in various animal models for urethral reconstruction.

The bladder submucosa matrix (60), proved to be a suitable graft for repair of urethral defects in rabbits. The neourethras demonstrated a normal urothelial luminal lining and organized muscle bundles These results were confirmed clinically in a series of patients with a history of failed hypospadias recons-

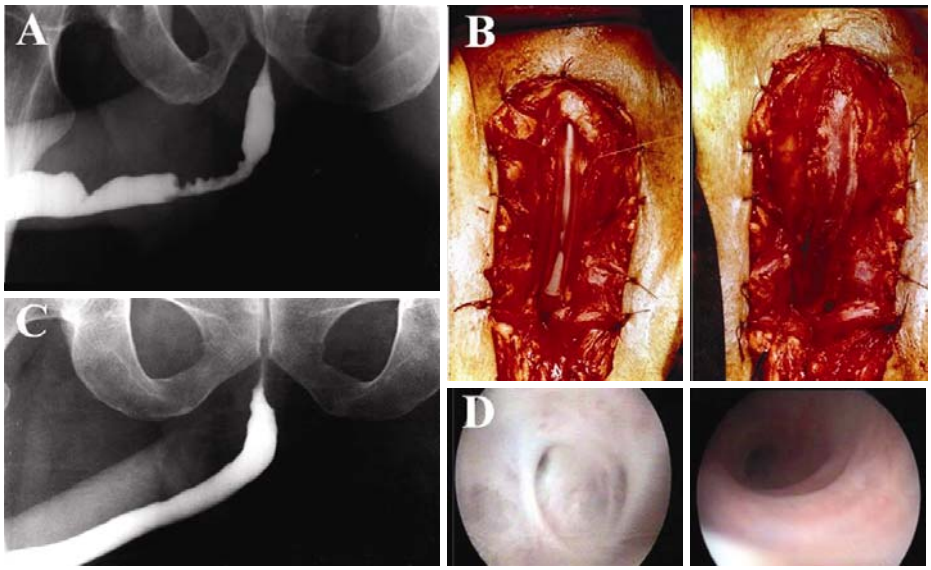

Figure 2 - Tissue engineering of the urethra using a collagen matrix.
A: Representative case of a patient with a bulbar stricture.
B: During the urethral repair surgery, strictured tissue is excised, preserving the urethral plate on the left side, and matrix is anastamosed to the urethral plate in an onlay fashion on the right. The boxes in both photos indicate the area of interest, including the urethra, which appears white in the left photograph. In the left photograph, the arrow indicates the area of stricture in the urethra. On the right, the arrow indicates the repaired stricture. Note that the engineered tissue now obscures the native white urethral tissue in an onlay fashion in the right photograph.
C: Urethrogram 6 months after repair.
D: Cystoscopic view of urethra before surgery on the left side, and 4 months after repair on the right side.

truction wherein the urethral defects were repaired with human bladder acellular collagen matrices (fig. 2) (17). The neourethras were created by anastomosing the matrix in an onlay fashion to the urethral plate. The size of the created neourethra ranged from 5 to 15 cm. After a 3-year follow-up, three of the four patients had a successful outcome in regard to cosmetic appearance and function. One patient who had a 15-cm neourethra created developed a subglanular fistula. The acellular collagen-based matrix eliminated the necessity of performing additional surgical procedures for graft harvesting, and both operative time and the potential morbidity from the harvest procedure were decreased. Similar results were obtained in pediatric and adult patients with primary urethral stricture disease using the same collagen matrices (76, 77). Today, more than 200 pediatric and adult patients with urethral disease have been successfully treated in an onlay manner with a bladder-derived collagen-based matrix.
The above techniques, using non-seeded acellular matrices, were applied experimentally and clinically in a successful manner for onlay urethral repairs. However, when tubularized urethral repairs were attempted experimentally, adequate urethral tissue regeneration was not achieved, and complications ensued, such as graft contracture and stricture formation (78). Autologous rabbit bladder epithelial and smooth muscle cells were grown and seeded onto pre-configured tubular matrices. Entire urethra segments were resected and urethroplasties were performed with tubularized collagen matrices either seeded with cells, or without cells. The tubularized collagen matrices seeded with autologous cells formed new tissue which was histologically similar to native urethra. The tubularized collagen matrices without cells lead to poor tissue development, fibrosis, and stricture formation. These findings were confirmed clinically. A clinical trial using tubularized non-seeded SIS for urethral stricture repair was performed in 8 evaluable patients. Two patients with short inflammatory strictures maintained urethral patency. Stricture recurrence developed in the other 6 patients within 3 months of surgery (79).
Thus, non-seeded matrices are able to replace urethral segments when used in an onlay fashion because of the short distances required for tissue ingrowth. However, if a tubularized urethral repair is needed, the matrices need to be seeded with autologous cells in order to avoid the risk of stricture formation and poor tissue development.
Most recently, Raya-Rivera and colleagues were able to show that synthetic biomaterials can also be used in urethral reconstruction when they are tubularized and seeded with autologous cells (80). This group used polygycolic acid:poly(lactide-co-glycolic acid) scaffolds seeded with autologous cells derived from bladder biopsies taken from each patient. The seeded scaffolds were then used to repair urethral defects in 5 boys. Upon follow-up, it was found that most of the boys had excellent urinary flow rates post-operatively, and voiding cystourethrograms indicated that these patients maintained wide urethral calibers. Urethral biopsies revealed that the grafts had developed a normal appearing architecture consisting of urothelial and muscular tissue.

Bladder

Over the last few decades, several bladder wall substitutes have been attempted with both synthetic and organic materials. Synthetic materials that have been tried in experimental and clinical settings include polyvinyl sponge, Teflon, collagen matrices, Vicryl (PGA) matrices, and silicone. Most of these attempts have failed because of mechanical, structural, functional, or biocompatibility problems. Usually, permanent synthetic materials used for bladder reconstruction succumb to mechanical failure and urinary stone formation, and use of degradable materials leads to fibroblast deposition, scarring, graft contracture, and a reduced reservoir volume over time (19, 81). Because of this, there has been an increase in the use of various collagen-based matrices for tissue regeneration. Non-seeded allogeneic acellular bladder matrices have served as scaffolds for the ingrowth of host bladder wall components. The matrices are prepared by mechanically and chemically removing all cellular components from bladder tissue (24, 59, 61, 82, 83). The matrices serve as vehicles for partial bladder regeneration, and relevant antigenicity is not evident. However, in multiple studies using various materials as non-seeded grafts for cystoplasty, the urothelial layer was able to regenerate normally, but the muscle layer, although present, was not fully developed (24, 61, 82, 84-86).
Thus, it was hypothesized that building the three-dimensional structure constructs in vitro, before implantation, would facilitate the eventual terminal differentiation of the cells after implantation in vivo and would minimize the inflammatory response toward the matrix, thus avoiding graft contracture and shrinkage. Cell-seeded allogeneic acellular bladder matrices were used for bladder augmentation in dogs (24). The regenerated bladder tissues contained a normal cellular organization consisting of urothelium and smooth muscle and exhibited a normal compliance. The bladders showed a significant increase (100%) in capacity when augmented with scaffolds seeded with cells, compared to scaffolds without cells (30%).
To better address the functional parameters of tissue-engineered bladders, another dog model was designed that required a subtotal cystectomy with subsequent replacement

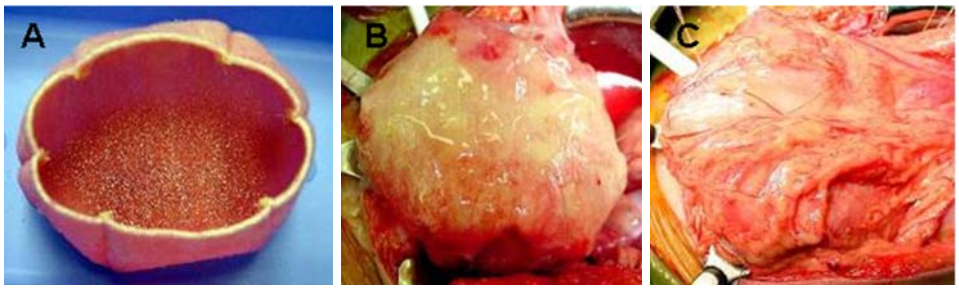

Figure 3 - Construction of engineered bladder.
A: Scaffold material seeded with cells for use in bladder repair.
B: The seeded scaffold is anastamosed to native bladder with running 4-0 polyglycolic sutures.
C: Implant covered with fibrin glue and omentum.

with a tissue-engineered organ (27). Cystectomy-only and non-seeded controls maintained average capacities of 22% and 46% of preoperative values, respectively. An average bladder capacity of 95% of the original precystectomy volume was achieved in the cell-seeded tissue engineered bladder replacements. These findings were confirmed radiographically. The subtotal cystectomy reservoirs that were not reconstructed and the polymer-only reconstructed bladders showed a marked decrease in bladder compliance (10% and 42% total compliance). The compliance of the cell-seeded tissue-engineered bladders showed almost no difference from preoperative values that were measured when the native bladder was present (106%).

A clinical experience involving engineered bladder tissue for cystoplasty reconstruction was conducted starting in 1998. A small pilot study of seven patients was reported, using a collagen scaffold seeded with cells either with or without omentum coverage, or a combined PGA-collagen scaffold seeded with cells and omental coverage (fig. 3). The patients reconstructed with the engineered bladder tissue created with the PGA-collagen cell-seeded scaffolds with omental coverage showed increased compliance, decreased end-filling pressures, increased capacities and longer dry periods over time (fig. 4) (87). It is clear from this experience that the engineered bladders continued their improvement with time, mirroring their continued development. Although the experience is promising in terms of showing that engineered tissues can be implanted safely, it is just a start in terms of accomplishing the goal of engineering fully functional bladders. FDA Phase 2 studies have now been completed, however, and indicate that this technology may be useful in patients with neurogenic bladder secondary to either spina bifida or spinal injury.

Genital tissues and organs

Reconstructive surgery is required for a wide variety of pathologic penile conditions, including penile carcinoma, trauma, severe erectile dysfunction, and congenital conditions such as ambiguous genitalia, hypospadias, and epispadias. One of the major limitations of phallic reconstructive surgery is the availability of sufficient autologous tissue. Nongenital autologous tissue sources have been used for decades. Phallic reconstruction was initially attempted in the late 1930s, with rib cartilage used as a stiffener for patients with traumatic penile loss (88, 89), but this process produced unsatisfactory functional and cosmetic results. Silicone rigid prostheses were popularized in the 1970s and have been used widely (90, 91). However, biocompatibility issues have been a problem in selected patients (92, 93).

Phallic reconstruction with autologous tissue, derived from the patient's own cells, may be preferable in selected cases. One of the major components of the phallus is corporal smooth muscle. Initial experiments have shown that cultured human corporal smooth muscle cells may be used in conjunction with biodegradable polymers to create corpus cavernosum tissue de novo (94). In order to look at the functional parameters of the engineered corpora, acellular corporal collagen matrices were obtained from donor rabbit penis and autologous corpus cavernosal smooth muscle and endothelial cells were harvested, expanded and seeded on the matrices. An entire cross-sectional segment of protruding rabbit phallus was excised, leaving the urethra intact. Cell seeded matrices were interposed into the excised corporal space. Functional and structural parameters (cavernosography, cavernosometry, mating behavior and sperm ejaculation) were followed, and histological, immunocytochemical and Western blot analyses were performed up to 6 months after implantation. The engineered corpora cavernosa achieved adequate structural and functional parameters (95). This technology was further confirmed when the entire rabbit corpora was removed and replaced with the engineered scaffolds. Most interestingly, mating activity in the animals with the engineered corpora appeared normal by 1 month after implantation. The presence of sperm was confirmed during mating, and was present in all the rabbits with the engineered corpora. The female rabbits mated with the animals implanted with engineered corpora and they conceived and delivered healthy pups. These studies demonstrate that penile corpora cavernosa tissue can be engineered. Further studies will be needed to confirm the long-term functionality of these organs. In addition, further studies are needed to show that comparable human structures can also be engineered.

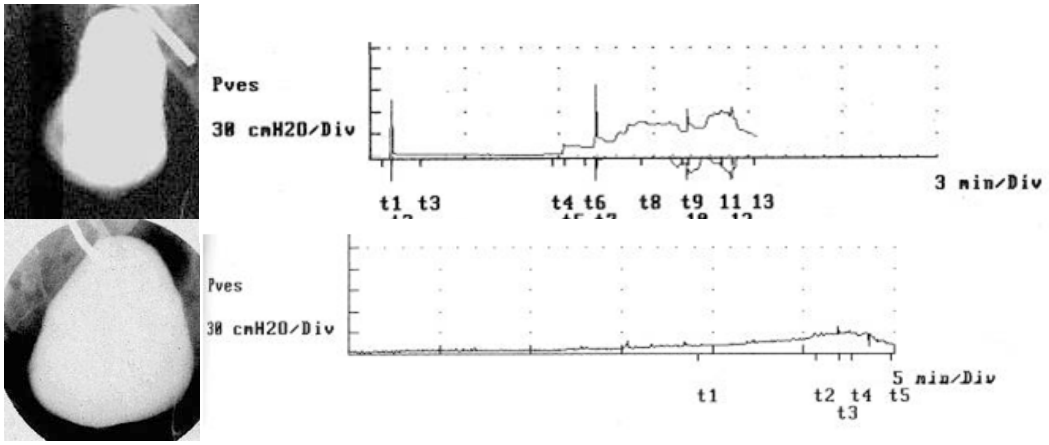

Figure 4 - Cystograms and urodynamic studies of a patient before and after implantation of the tissue engineered bladder. A: Preoperative results indicate an irregular-shaped bladder in the cystogram (left) and abnormal bladder pressures as the bladder is filled during urodynamic studies (right).
B: Postoperatively, findings are significantly improved.

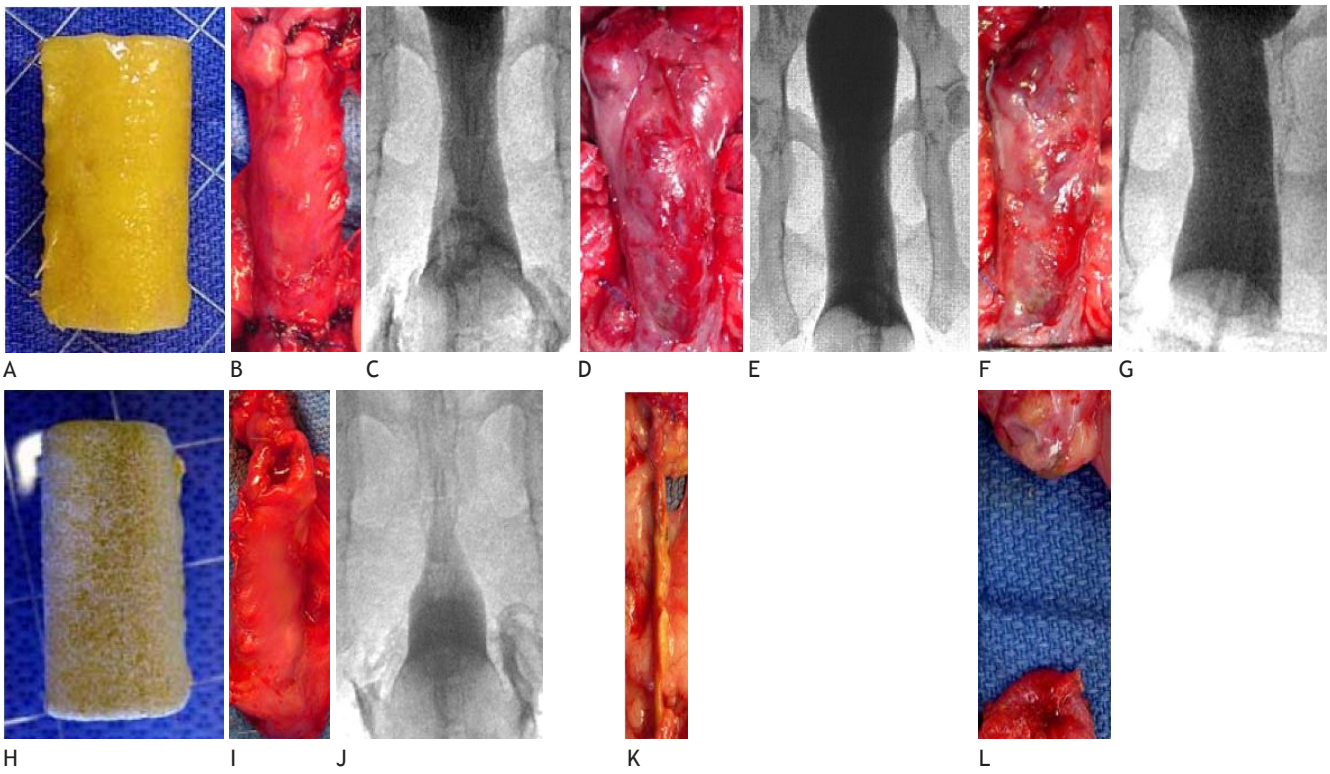

Figure 5 - Appearance of tissue engineered neo-vaginas.
A: Tubular polymer scaffold after cell seeding and 1 week in vitro culture, prior to implantation in vivo.
B, D, and F: Indicate gross appearance.
C, E, and G: Show vaginography of cell-seeded constructs 1, 3,and 6 months post-implantation, respectively.
H: Unseeded control scaffold prior to implantation.
I, K, and L: Gross appearance of unseeded construct at 1, 3, and 6 months post-implantation.
J: Vaginography of unseeded graft at 1 month.

Female Genital and Reproductive Tissues

Congenital malformations of the uterus may have profound implications clinically. Patients with cloacal exstrophy and intersex disorders may not have sufficient uterine tissue present for future reproduction. We investigated the possibility of engineering functional uterine tissue using autologous cells (96). Autologous rabbit uterine smooth muscle and epithelial cells were harvested, then grown and expanded in culture. These cells were seeded onto pre-configured uterine-shaped biodegradable polymer scaffolds, and the constructs were used for subtotal autologous uterine tissue replacement. Six months after implantation, analyses confirmed the presence of uterine tissue components. Biomechanical analyses and organ bath studies showed that the functional characteristics of the engineered tissues were similar to those of normal uterine tissue. Breeding studies using these engineered uteri are currently being performed.
Similarly, several pathologic conditions, including congenital malformations and malignancy, can adversely affect normal vaginal development or anatomy. Vaginal reconstruction has traditionally been challenging due to the paucity of available native tissue. Acellular materials have been used experimentally for vaginal reconstruction in rats (97). The feasibility of using cells to engineer vaginal replacements was also investigated (98). Vaginal epithelial and smooth muscle cells of female rabbits were harvested, grown, and expanded in culture. These cells were seeded onto biodegradable polymer scaffolds, and the cell-seeded constructs were then implanted into mice. Functional studies in the tissue-engineered constructs showed similar properties to normal vaginal tissue. When these constructs were used for autologous total vaginal replacement in a rabbit model, patent functional vaginal structures were noted in the tissue-engineered specimens, while the non-cell-seeded structures were noted to be stenotic (99) (fig. 5) These studies indicated that a regenerative medicine approach to clinical vaginal reconstruction would be a realistic possibility. Clinical trials are currently being conducted.

Kidney

Although the kidney was the first organ to be substituted by an artificial device and the first successfully transplanted organ (2), current modalities of treatment are far from satisfactory. Renal tissue is arguably one of the most difficult tissues to replicate in the laboratory. The kidney is very complex and the unique structural and cellular heterogeneity present within it creates many challenges. The system of nephrons and collecting ducts within the kidney is composed of multiple functionally and morphologically distinct segments. For this reason, appropriate conditions must be provided to ensure the long-term survival, differentiation and growth of many types of cells at once. Efforts in the area of kidney tissue regeneration have focused on the development of a reliable cell source (100-105). Moreover, optimal growth conditions have been extensively investigated to provide adequate enrichment to achieve stable renal cell expansion systems (106-109).
Isolation of particular cell types that produce specific factors may be a good approach for selective cell therapies for renal failure. For example, cells that produce erythropoietin have been isolated in culture, and these cells could eventually be used to treat anemia that results from end stage renal failure (110). Other more ambitious approaches tackle the goal of total renal function replacement. To create kidney tissue that would deliver full renal function, a culture containing all of

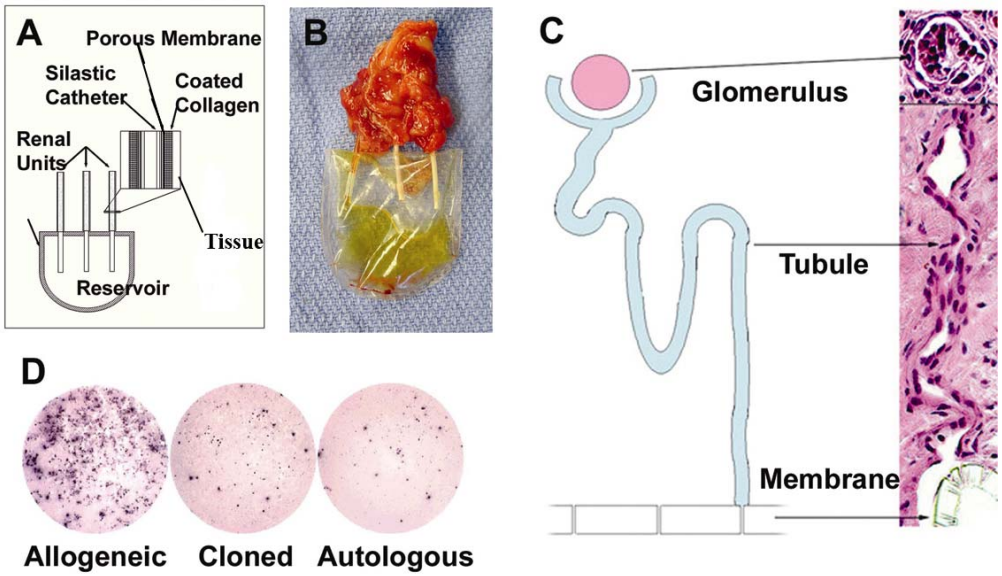

Figure 6 - Combining therapeutic cloning and tissue engineering to produce kidney tissue.
A: Illustration of the tissue-engineered renal unit.
B: Renal unit seeded with cloned cells, three months after implantation, showing the accumulation of urine-like fluid.
C: Clear unidirectional continuity between the mature glomeruli, their tubules, and silastic catheter.
D: Elispot analyses of the frequencies of T cells that secrete IFN□ after stimulation with allogeneic renal cells, cloned renal cells, or nuclear donor fibroblasts. Cloned renal cells produce fewer IFN□ spots than the allogeneic cells, indicating that the rejection response to cloned cells is diminished. The presented wells are single representatives of duplicate wells.

the cell types comprising the functional nephron units should be used. Optimal culture conditions to nurture renal cells have been extensively studied and cells grown under these conditions have been reported to maintain their cellular characteristics (111). Cells obtained through the initial process of nuclear transfer were retrieved and expanded from cloned tissue. Moreover, renal cells placed in a three-dimensional culture environment are able to reconstitute into renal structures.
In one study, mouse renal cells were harvested, expanded in culture, and seeded onto a tubular device constructed from polycarbonate (112). The tubular device was connected at one end to a silastic catheter which terminated into a reservoir. The device was implanted subcutaneously in athymic mice. Histological examination of the implanted device demonstrated extensive vascularization as well as formation of glomeruli and highly organized tubule-like structures. Immunocytochemical staining with anti-osteopontin antibody, which is secreted by proximal and distal tubular cells and the cells of the thin ascending loop of Henle, stained the tubular sections. Immunohistochemical staining for alkaline phosphatase stained proximal tubule-like structures. Uniform staining for fibronectin in the extracellular matrix of newly formed tubes was observed. The fluid collected from the reservoir was yellow and contained 66 mg/dl uric acid (as compared to 2mg/dl in plasma) suggesting that these tubules are capable of unidirectional secretion and concentration of uric acid. The creatinine assay performed on the collected fluid showed an 8.2 fold increase in concentration, as compared to serum. These results demonstrated that single cells form multicellular structures can become organized into functional renal units that are able to excrete high levels of solutes through a urine-like fluid (112).
To determine whether renal tissue could be formed using an alternative cell source, nuclear transplantation (therapeutic cloning) was performed to generate histocompatible tissues, and the feasibility of engineering syngeneic renal tissues *in vivo* using these cloned cells was investigated (fig. 6) (111). Nuclear material from bovine dermal fibroblasts was transferred into unfertilized enucleated donor bovine eggs. Renal cells from the cloned embryos were harvested, expanded *in vitro*, and seeded onto three-dimensional renal devices. The devices were implanted into the back of the same steer from which the cells were cloned, and were retrieved 12 weeks later. This process produced functioning renal units. Urine production and viability were demonstrated after transplantation back into the nuclear donor animal. Chemical analysis suggested unidirectional secretion and concentration of urea nitrogen and creatinine. Microscopic analysis revealed formation of organized glomeruli and tubular structures. Immunohistochemical and RT-PCR analysis confirmed the expression of renal mRNA and proteins. These studies demonstrated that cells derived from nuclear transfer can be successfully harvested, expanded in culture, and transplanted *in vivo* with the use of biodegradable scaffolds on which the single suspended cells can organize into tissue structures that are genetically identical to that of the host. These studies were the first demonstration of the use of therapeutic cloning for regeneration of tissues *in vivo*.

Blood vessels

Xenogenic or synthetic materials have been used as replacement blood vessels for complex cardiovascular lesions. However, these materials typically lack growth potential, and may place the recipient at risk for complications such as stenosis, thromboembolization, or infection (113). Tissue-engineered vascular grafts have been constructed using autologous cells and biodegradable scaffolds and have been applied in dog and lamb models (114-117). The key advantage of using these autografts is that they degrade *in vivo*, and thus allow the new tissue to form without the long term presence of foreign material (113). Application of these techniques from the laboratory to the clinical setting has begun, with autologous vascular cells harvested, expanded, and seeded onto a biodegradable scaffold (118). The resultant autologous construct was used to replace a stenosed pulmonary artery that had been previously repaired. Seven months after implantation, no evidence of graft occlusion or aneurysmal changes was noted in the recipient.

Heart

In the United States, over 5 million people currently live with some form of heart disease, and many more are diagnosed each year. While many medications have been developed to assist the ailing heart, the treatment for end-stage heart failure still remains transplantation. Unfortunately, as with other organs, donor hearts are in short supply, and even when a transplant can be performed, the patient must endure the side effects created by lifelong immunosuppression. Thus, alternatives are desperately needed, and the development of novel methods to regenerate or replace damaged heart muscle using tissue engineering and regenerative medicine techniques is an attractive option.
Cell therapy for infracted areas of the heart is attractive, as these methods involve a rather simple injection into the dam-

aged area of a patient's heart, rather than a rigorous surgical procedure, to complete. Various types of stem cells have been investigated for their potential to regenerate damaged or dead heart tissue in this manner. Skeletal muscle cells, bone marrow stem cells (both mesenchymal and hematopoietic), amniotic fluid stem cells, and embryonic stem cells have been used for this purpose. In this technique, cells are suspended in a biocompatible matrix that can range from simple normal saline to complex yet biocompatible hydrogels depending on the type of injection to be performed. The cells are either injected into the damaged area of the heart itself, or they are injected into the coronary circulation with the hope that they will home to the damaged area, take up residence there, and begin to repair the tissue. However, injectable therapies have been shown to be relatively inefficient, and cell loss is quite substantial. Newer methods of tissue engineering include the development of engineered "patches," which are comprised of cells adhered to a biomaterial, that can theoretically be used to replace the damaged area of the heart. These techniques have promise, but require further research into the optimal cell types and biomaterials for this purpose before they can be used extensively in the clinic (see [119]for an excellent review of these methods).

However, the methods described above could only be used in cases where a relatively small section of heart muscle was damaged. In cases where a large area or even the whole heart has become nonfunctional, a more radical approach may be required. In these situations, the use of a bioartificial heart would be ideal, as rejection would be avoided and the problems associated with a mechanical heart (such as thromboembolus formation) would be abolished. To this end, Ott et. al. recently developed a novel heart construct in vitro using decellularized cadaveric hearts. By reseeding the tissue scaffold that remained after a specialized decellularization process with various types of cells that make up a heart (cardiomyocytes, smooth muscle cells, endothelial cells, and fibrocytes) and culturing the resulting construct in a bioreactor system designed to mimic physiologic conditions, this group was able to produce a construct that could generate pump function on its own (120). This study suggests that production of bioartificial hearts may one day be possible.

Liver

The liver can sustain a variety of insults, including viral infection, alcohol abuse, surgical resection of tumors, and acute drug-induced hepatic failure. The current therapy for liver failure is liver transplantation. However, this therapy is limited by the shortage of donors and the need for lifelong immunosuppressive therapy. Cell transplantation has been proposed as a potential solution for liver failure. This is based on the fact that the liver has enormous regenerative potential in vivo suggests that in the right environment, it may be possible to expand liver cells in vitro in sufficient quantities for tissue engineering (121). Many approaches have been tried, including development of specialized media, co-culture with other cell types, identification of growth factors that have proliferative effects on these cells, and culture on three-dimensional scaffolds within bioreactors (121).

Recently, Baptista and colleagues described the fabrication of three-dimensional, naturally derived liver scaffolds with an intact vascular tree {Baptista, 2011 #1481}. Livers from different species were perfused with a detergent solution to remove the cellular components of the tissue while preserving the extracellular matrix components and the intact vascular network. The decellularized vascular network was able to withstand fluid flow, and fluid that entered through a central inlet vessel was shown to branch into the extensive capillary bed and then exit the construct through a single outlet vessel. This functional vascular network was used to reseed the scaffolds with human fetal liver and endothelial cells, which engrafted in their putative native locations within the decellularized organ and displayed typical endothelial, hepatic, and biliary epithelial markers, thus creating a liver-like tissue in vitro. These results represent a significant advancement in the bioengineering of whole organs. This technology may provide the necessary tools to produce the first fully functional bioengineered livers for organ transplantation.

Trachea

Few treatment options are currently available for patients who suffer from severe congenital tracheal pathology, such as stenosis, atresia, and agenesis, due to the limited availability of autologous transplantable tissue in the neonatal period. Tissue engineering in the fetal period may be a viable alternative for the surgical treatment of these prenatally diagnosed congential anomalies, as cells could be harvested and grown into transplantable tissue in parallel with the remainder of gestation. Chondrocytes from both elastic and hyaline cartilage specimens have been harvested from fetal lambs, expanded *in vitro*, then dynamically seeded onto biodegradable scaffolds (122). The constructs were then implanted as replacement tracheal tissue in fetal lambs. The resultant tissue-engineered cartilage was noted to undergo engraftment and epithelialization, while maintaining its structural support and patency. Furthermore, if native tracheal tissue is unavailable, engineered cartilage may be derived from bone marrow-derived mesenchymal progenitor cells as well (123).

While exciting, the technique described above cannot produce large segments of functional airway, and thus, it is not applicable for adults who need tracheal repair or replacement due to injury or disease. However, in December 2008, Birchall's group reported the construction and implantation of the first tissue-engineered human trachea (124). In order to produce a scaffold on which to engineer the replacement trachea, this group removed all of the cells and MHC antigens from a human donor trachea. The resulting acellular matrix was then reseeded with epithelial cells and mesenchymal stem-cell-derived chondrocytes that had been obtained from a 30-year old woman with end-stage bronchomalacia resulting from tuberculosis. This tissue-engineered graft was then used to replace the woman's diseased left main bronchus. The group reported that immediately after surgery, the graft began to serve as a functional airway and produced an improvement in the patient's quality of life. At four months after surgery, the engineered graft appeared normal in all respects. Importantly, the patient had not developed anti-donor antibodies and was not on any type of immunosuppressive medication. However, the production of this tissue engineered trachea took nearly 3 months in the laboratory, which meant that this technique was not feasible for patients with an urgent need for transplantation. Last year, the same group reported that they had decreased the graft production time to just over 3 weeks by improving the decellularization process for the donor tracheal tissue, which suggests that with more research, tissue engineered tracheas may become more widely used in the clinic (125).

Summary and conclusions

Regenerative medicine efforts are currently underway experimentally for virtually every type of tissue and organ within the human body. As regenerative medicine incorporates the fields of tissue engineering, cell biology, nuclear transfer, and materials science, personnel who have mastered the techniques of cell harvest, culture, expansion, transplantation, as well as polymer design are essential for the successful application of these technologies to extend human life. Various tissues are at different stages of development, with some

already being used clinically, a few in preclinical trials, and some in the discovery stage. Recent progress suggests that engineered tissues may have an expanded clinical applicability in the future and may represent a viable therapeutic option for those who would benefit from the life-extending benefits of tissue replacement or repair.

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