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il punto

Tissue engineering technologies: just a quick note about transplantation of bioengineered donor trachea and augmentation cystoplasty by *de novo* engineered bladder tissue

C. ALBERTI

SUMMARY: **Tissue engineering technologies: just a quick note about transplantation of bioengineered donor trachea and augmentation cystoplasty by *de novo* engineered bladder tissue.**

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Tissue engineering is a multidisciplinary scientific field that aims at manufacturing in vitro biological substitutes to enhance or replace failing human organs. Various types of biodegradable synthetic polymer (polyglycolic acid, PGA; polylactic acid, PLA; polylactic-coglycolic acid), naturally-derived (alginate, collagen), acellular tissue-made up (small intestinal submucosa, SIS; acellular bladder submucosa, ABS) and composite (PGA bound to collagen) materials have been used as scaffold for either "unseeded" (cell-free) or "seeded" (autologous cells seeded onto the matrix) tissue engineering strategies. The unseeded technique is directed at promoting the in vivo tissue regenerative process, unfortunately with certain limitations, whereas the "seeded technique" aims at creating in vitro functional replacement tissues or organs. Recently, a decellularized human dead donor trachea has been used as scaffold, that was then seeded, in vitro, by recipient epithelial cells and mesenchymal stem cell-derived chondrocytes, to obtain a bioengineered airway to replace recipient's failing left main bronchus. As far as clinical applications in Urology are concerned, a cell-based approach (PGA-collagen composite scaffold seeded with autologous cells) has been achieved to successfully carry-out an augmentation cystoplasty in subjects with end-stage neuropathic high pressure/poorly compliant bladder. The use of autologous cells, wherein a specimen of tissue is harvested by biopsy from the host, avoids the risk of rejection. Nevertheless, the use of adult organ-specific cells shows many limitations, such as difficulties in their harvesting (potential complications associated with invasive biopsies) and their low proliferative ability. Therefore, various populations of either embryonic or adult stem cells and progenitor cells have been studied as useful cell sources for the tissue engineering. Bioreactors are essential in such technologies, both providing chemo-physical cell culture dynamic conditions, that mimic the in vivo environment, and allowing the assessment of responses of biological substitutes to different biochemical signals and mechanical forces.

RIASSUNTO: **Tecnologie di ingegneria tessutale: breve nota su trapianto di trachea da donatore bioingegnerizzata e cistoplastica di ampliamento mediante tessuto vescicale ingegnerizzato *de novo*.**

C. ALBERTI

Per ingegneria dei tessuti si intende un settore scientifico multidisciplinare volto alla realizzazione in vitro di strutture biologiche di ricambio, atte a rinforzare o sostituire organi umani in precarie condizioni. Diversi tipi di materiali – quali polimeri sintetici biodegradabili (acido poliglicolico, PGA; acido polilattico, PLA; copolimero PGA-PLA), sostanze di derivazione naturale (alginato, collagene), matrici acellulari (submucosa del piccolo intestino, SIS; submucosa acellulare vescicale, ABS), o tra loro assemblati (PGL legato a collagene) – sono stati impiegati come intelaiature (scaffold) sia prive di cellule ("unseeded technique"), allo scopo di promuovere, in vivo, nell'organo in cui vengono impiantate, naturali processi rigenerativi, non sempre, però, attuantisi, sia per sviluppare, in vitro, previa semina, nella loro compagine, di cellule del potenziale ricevente (autologous cell seeded technique), un tessuto ingegnerizzato, idoneo all'impiego in chirurgia ricostruttiva. Recentemente, la trachea di donatore morto, opportunamente decellularizzata, è stata usata quale scaffold per essere seminata, in vitro, con cellule epiteliali e condrociti derivati da cellule staminali mesenchimali del ricevente, al fine di ottenere un segmento ingegnerizzato di via respiratoria tale da sostituire il grosso bronco sinistro, gravemente malacico, del ricevente. In riferimento alle applicazioni in ambito urologico, è stato coronato da successo l'impiego di una "seeded technique" (cellule autologhe seminate su scaffold composto di PGA legato a collagene) per confezionare tessuto ingegnerizzato vescicale usato nella realizzazione di cistoplastica d'ampliamento in soggetti affetti da "vescica neurologica", ormai in fase estremamente grave (livelli alti di pressione endovescicale, compliance vescicale molto ridotta). L'impiego di cellule autologhe, ottenute, mediante biopsia, dai tessuti del ricevente, evita il rischio di rigetto dopo l'impianto. Peraltro, il ricorso a cellule organo-specifiche "adulte" presenta dei limiti correlati alla loro raccolta (potenziali complicazioni associate all'invasività della biopsia) ed alle difficoltà di cultura in vitro data la loro bassa capacità proliferativa. Pertanto, sono stati presi in considerazione diversi tipi di cellule staminali, embrionali o adulte, e di cellule progenitrici, al fine di utilizzarle in ingegneria dei tessuti. Per queste tecnologie risulta indispensabile l'impiego di bioreattori, atti a riprodurre, nelle culture cellulari, le condizioni dinamiche chimico-fisiche proprie dell'ambiente tissutale naturale, ed a consentire la valutazione delle risposte delle strutture bioingegnerizzate a differenti segnali biochimici e forze meccaniche.

Libero Docente di Semeiotica Chirurgica
Università degli Studi di Parma, Italy

KEY WORDS: Tissue engineering - Trachea - Bladder - Stem cells - Reconstructive Surgery - Urology.
Ingegneria tissutale - Trachea -Vescica - Cellule staminali - Chirurgia ricostruttiva - Urologia.

Regenerative medicine is a fast-evolving polydisciplinary scientific field – including materials science, cell culture, therapeutic cloning, stem cells and engineering – that aims at manufacturing functional biological structures to regenerate and enhance failing organs or create biological replacement substitutes (1-6).

Tissue engineering technologies may use as scaffold either biodegradable synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polylactic-coglycolic acid, sometimes bound to collagen matrix (composite scaffold), or naturally-derived materials like alginate and collagen or, moreover, acellular tissue matrices as small intestinal submucosa (SIS) and acellular bladder submucosa (ABS). Current strategies include either *unseeded* or *seeded* techniques, the first directed to promote, by incorporating a cell-free scaffold into a diseased organ, the *in vivo* tissue regenerative process (regenerative medicine), whereas the second, by using recipient cells seeded onto a scaffold, aims at generating, *in vitro*, functional replacement tissues or organs, thus carrying out a properly named tissue engineering (7-14). Just intriguingly, a decellularized donor organ (trachea) has been recently used as ready made organ-shaped matrix scaffold to be seeded with recipient cells, so that to remplace a damaged organ (bronchus) (15, 16). Such technique is here compared with the tissue engineering using a cell-seeded composite scaffold to carry-out an augmentation cystoplasty (17, 18).

Transplantation of bioengineered donor trachea

Over the last two decades, many attemps have been carried out to construct tracheal spare parts to be used in different tracheal pathological conditions such as either congenital abnormalities (atresia, fistulas) or acquired diseases (chronic inflammatory stenosis, tumor invasion, etc), but they have met with serious difficulties (15, 16, 19-21).

In an animal model, a tissue-engineered tracheal equivalent – tubular cartilage tissue lined with recipient nasal epithelial cells – has been manufactured but unfortunately it proved to be easily collapsable (22, 23).

In other animal experimentations, donor tracheal matrix, obtained by decellularizing the native donor organ with a detergent-enzymatic (deoxyribonuclease, DNase) treatment to decrease its major histocompatibility complex (MHC)antigens, was able to support *in vitro* the adhesion and growth of both chondrocytes and tracheal epithelial cells harvested from recipient animal, thus proposing an alternative approach to repair or replace tracheal defects (24, 25).

The first *clinical* application of a *bioengineered airway patch*, made from autologous muscle cells and fibroblasts seeded onto collagen-matrix, has been successfully carried out to repair an airway defect, measuring 2x2 cm, occurring at the anastomotic site after carinal pneumonectomy and, most interestingly, such tissue-engineered patch was reseeded *in vivo* by ciliated respiratory epithelium (16). Subsequently, a transplantation of *bioengineered airway segment* has been performed in 30-year-old woman with post-tubercolous chronic tracheitis and left main bronchus end-stage malacia, by using, as a ready made organ-schaped scaffold, a human dead donor trachea. This explant, at first, was decellularized by a detergent-enzymatic method, to achieve the complete removal of donor MHC antigens and preserve only the underlying connective matrix, which then was seeded *in vitro* with recipient mesenchymal stem cell-derived chondrocytes and epithelial cells, so that to obtain a living tissue construct to replace the recipient's diseased left main bronchus; the graft straight-away provided the patient with a functional airway, free from the risks of rejection (Table 1) (15).

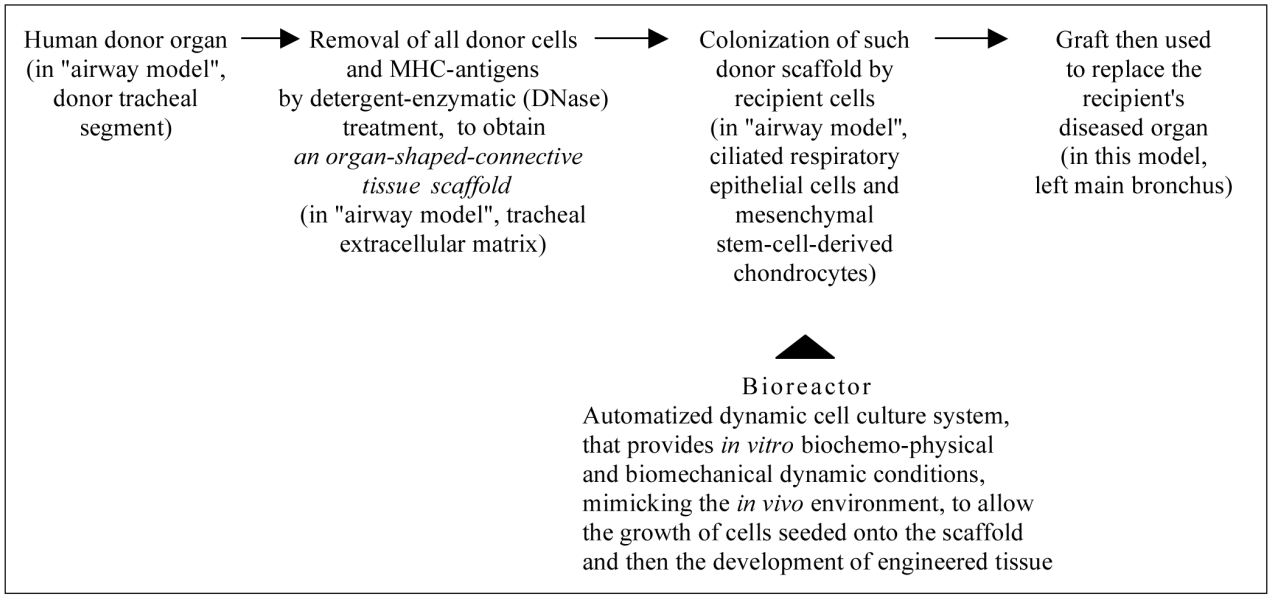
Apart from that, in the field of experimental heart bioengineering, many attemps have been performed to similarly obtain a *tissue-engineered heart*, by utilizing, in animal models, a decellularized donor heart, whose preserving the underlying extracellular matrix and reseeding it with cardiac/endothelial cells; the function of such construct has been then tested in a bioreactor to simulate the cardiac physiology (26, 27).

Bioreactors, also named biomimetic reactors, are essential in tissue engineering technologies not only because they provide chemo-physical *in vitro* cell-culture dynamic conditions (O_2 and CO_2 levels, pH, humidity, temperature, efficient nutrition, waste removal) simulating the *in vivo* environment, but also because they enable a proper assessment of the responses of biological substitutes – cell activity, differentiation and function – to various chemo-physical signals and different mechanical strains (27-30).

Augmentation cystoplasty by *de novo* engineered bladder tissue

Congenital abnormalities and acquired diseases may lead to bladder damage or loss, hence requiring aug-

TABLE 1 - TRANSPLANTATION OF THE BIOENGINEERED DONOR ORGAN ("AIRWAY MODEL").



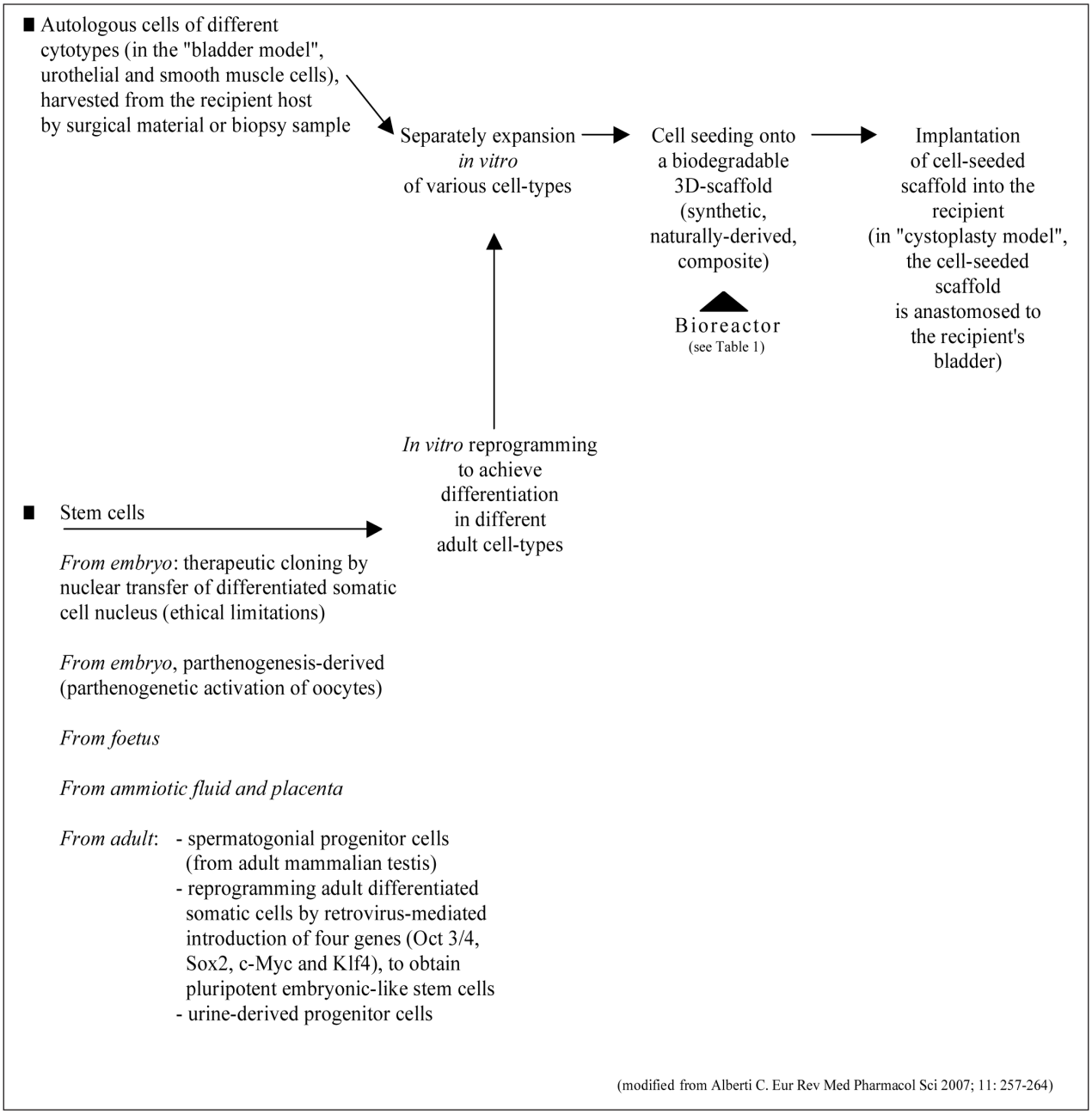
mentation or replacement bladder reconstructive surgery, whose gold standard still remains the use of bowel. However to prevent the problematic outcomes of the intestinal prostheses systemic metabolic derangements and/or pathomorphosic malignancies, particularly at the uretero-intestinal anastomoses and, moreover, considering that the prosthetic use of intestinal segments must be avoid in some conditions such as the short gut syndrome and chronic inflammatory bowel disease, research has been turned, over the last decades, on the development of new strategies for more proper bladder reconstruction. The at the end of past century emerging bladder tissue-engineering technologies include both *unseeded* and *seeded* strategies, but, unfortunately, the cell free matrices, rather than promote, in animal models, a natural *in vivo* vesical wall regenerative process, showed , at just one year of follow-up, a graft shrinkage (increased wall collagen deposition, disorganized muscle development, fibrosis) with bladder inadequate compliance (2, 7, 8, 10-12). The *autologous cell seeded tissue engineering technology*, instead, allows the development of a bladder tissue that is able to mimic the functional ability of smooth muscle wall together with displaying both barrier and sensory transducer urothelial properties (Table 2). In a canine cystoplasty model – subtotal trigone-sparing cystectomy followed by wall replacement with such engineered tissue – histological and immunocytochemical analyses showed a normally organized graft wall architecture, consisting of urothelial, suburothelial connective and smooth muscle layers, and *in vivo* dynamic cystograms and *in vitro* contractility studies had successful outcomes. Moreover, serum chemistry, complete blood count and electrolytes remained post-operatively within normal levels, thus ruling out the significant systemic toxicity (7, 8, 10, 32).

Even smooth muscle cells engineered from functionally abnormal extrophic and neuropathic bladder showed both morphologic and dynamic normal features, suggesting that smooth muscle cells from diseased organs can be engineered in a healthy tissue (13, 17, 18, 32)

Clinical validation of the concept of *de novo* bladder tissue engineering with autologous cell seeded technique – urothelial and smooth muscle recipient cells seeded onto a collagen-polyglicolic composite three dimensional scaffold – was successfully reached, by implanting such tissue engineered construct, with omental drap, in patients requiring augmentation cystoplasty because of end-stage neuropathic, myelomeningocele-related, high-pressure poorly compliant bladder. Such cystoplasty displayed normal trilayered architecture and both urodynamic and renal function parameters remained within physiologic ranges, on a follow-up of longer than 8 years (17, 18, 33).

Nevertheless, considering that the current techniques for tissue engineering depend upon a sample of autologous cells from the damaged recipient's organ, the specimens from a widely diseased organs may not yielt enough healthy cells. Furthermore, other limitations – such as potential complications associated with invasive tissue biopsies and low *in vitro* proliferative ability of adult organ-specific cells – have suggested the cell resort to other sources. Hence, promising chances of cell-based bladder engineering have been placed in the resort to pluri- and multipotent *stem cells*, that are capable of self-renewal

TABLE 2 - DE NOVO RECONSTITUTION OF A FUNCTIONAL HOLLOW ORGAN BY TISSUE ENGINEERING ("BLADDER MODEL").



and differentiation in several tissue-specific cell lineages (34-36). However, most current procedures to obtain pluripotent embryonic stem cells require the therapeuthic cloning by nuclear transfer and the their harvesting from blastocyst with embryo destruction, that meets with serious ethical issues. To avoid these problems, various intriguing pathways have been tried, looking to alternative sources of stem cells or progenitor cells: parthenogenesis-derived, from parthenogenetic activation of oocytes (37, 38); ammiotic fluid and placenta-extracted (39); spermatogonial progenitor cells, from adult testis (40); adult differentiated cell-derived stem cells, reprogramming somatic cells by retrovirus-mediated introduction of four genes (Oct3/4, Sox2, c-Myc and Klf4), thus obtaining the so-called *induced pluripotent stem cells*, iPS (41, 42); urine-derived progenitor cells with ability to differentiate into several bladder lineages such as urothelial, smooth muscle, endotelial, interstitial (43, 44).

Conclusions

The intriguing *transplantation of bioengineered donor trachea* concerns the use of a ready made organ-shaped matrix scaffold, that is obtained by removing, through a detergent-enzymatic treatment, every-type cells and MHC antigens from the donor organ, thus preserving the underlying extracellular matrix, which is seeded with recipient cells to develop, inside a proper bioreactor, a bioengineered organ to be implanted into the host (Table 1) (15, 16).

The tissue engineered bladder consists in a *de novo reconstitution of a functional tissue*, including the hard-working building of a three-dimensional scaffold, which is then seeded with recipient cells (Table 2) (17, 18, 33).

Both technologies, by using autologous cells to be seeded onto the anyway obtained scaffolds, allow to have available immunologically "personalized" tissue-engineered prostheses, therefore free from the risk of rejection after *in vivo* implantation, thus avoiding any immunosuppressive treatment.

Considering the dramatic advances, during little more than one decade, in the field of tissue engineering, there is to expect, looking to the near future, the achievement of further intriguing goals.

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