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editorial-review

Hollow organ tissue engineering: short updating about current approaches and forecast for major research advances

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Overall view

Regenerative medicine technologies are able to offer novel therapeutic modalities for patients suffering from diseased organs – end-stage organ failure, resected organs because of their either malignant or severe chronic inflammatory pathology, congenital organ abnormalities – which need organ replacement, though compared with an increasing shortage of donor organs (1-4).

Tissue engineering technologies, through combining biomaterial sciences and biotechnology by resorting to either therapeutic cloning or induced pluripotent stem cell (iPSC) supply (Table 1), can today provide the clinicians with intriguing regenerative medicine tools to repair tissues or replace organs (2, 3, 5). Bioengineered *non-modular organs* – such as bladder, skin, airways and vessels – are made of autologous cells seeded onto either natural or synthetic scaffold, without their own microvascular network, so getting, when implanted *in vivo*, nutrients and oxygen, by diffusion, from neighboring tissues, whereas bioengineered *modular organs*, organized in functioning units (so-called modules) – such as liver, kidney, pancreas, heart, small intestine – need their own vascular supply, however both construct types representing custom-made tissue engineered organs given the autologous origin of their cellular component, thus avoiding, for the recipient patients, a lifelong immunosuppressive treatment (4, 6, 7). Vascularized bioscaffolds of modular organs may be carried-out by decellularization of the donor organ while preserving its extracellular matrix with the vascular tree (4, 7, 8).

Essential tools to develop tissue engineered organs are specifically designed *bioreactors*, inside which the scaffold and cellular component are conditioned to biochemo-physical and mechanical dynamic conditions simulating those proper of organ to be replaced. In the field of the experimental bioreactor technology, to provide complex 3D-bioartificial tissue models with adequate nutrient supply, a biological vascularized carrier structure (scaffold) has been developed, by obtaining it from a decellularized small bowel segment in which the native microvascular network within the collagen matrix has been preserved (4, 9-11).

Intriguingly, the induction of mature somatic adult cells to de-differentiate into *pluripotent stem cells* (iPSC), that, in turn, may be conditioned to produce different specifically differentiated cell lineages, can allow limitless cell supplies for various tissue engineering applications (2, 3).

Tissue engineered bladder

The traditional intestinal neobladder exposes to the risk of pathomorphosic malignant transformation of the reservoir, particularly at the uretero-intestinal anastomoses because of preternatural connections between urothelium and intestinal epithelium, and can induce systemic metabolic imbalances (hyperchloremic acidosis, electrolyte and basic/acid derangements, fat maldigestion, nutritional deficiency) resulting from both the chronic exposure of intestinal mucosa to urinary components and the removal of the intestinal segment required for the bladder replacement. Other com-

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TABLE 1 - STEM CELL TYPES.

• *Totipotent*:	only peculiar to earliest stages of embryogenesis, are able to generate all three germ cell layers growing into the embryo together with extra-embryonic structures such as placenta.
• *Pluripotent*:	belong to this group both the naturally embryonic stem cells, that are able to generate all three germ cell layers growing into the embryo but not extra-embryonic structures, and the induced pluripotent embryonic-like stem cells ($iPSC_S$) obtained from mature somatic cells*. Nevertheless, the reprogramming process of somatic cells into $iPSC_S$ seems to compromise their genomic integrity (occurrence of genetic rearrangements and copy number variations), which is why their therapeutic use is temporarily unadvisable while carrying on, instead, with research about them (50).
• *Multipotent*:	later developed during the ontogenesis, are naturally able to generate only differentiated cell peculiar to their tissue of origin. Multipotent stem cells are present in all adult tissues, thus promoting their regeneration. Under certain conditions, they can produce mature cells not naturally present in their tissue of origin (transdifferentiation process due to plasticity properties).

** In this regard, even from certain adult mature cells it is now possible to directly induce the conversion to different mature cytotypes without the intermediate iPSC (e.g., human dermal fibroblasts, treated with OCT4 master control gene, can transdifferentiate directly into multilineage blood cell progenitors - ref. 51).*

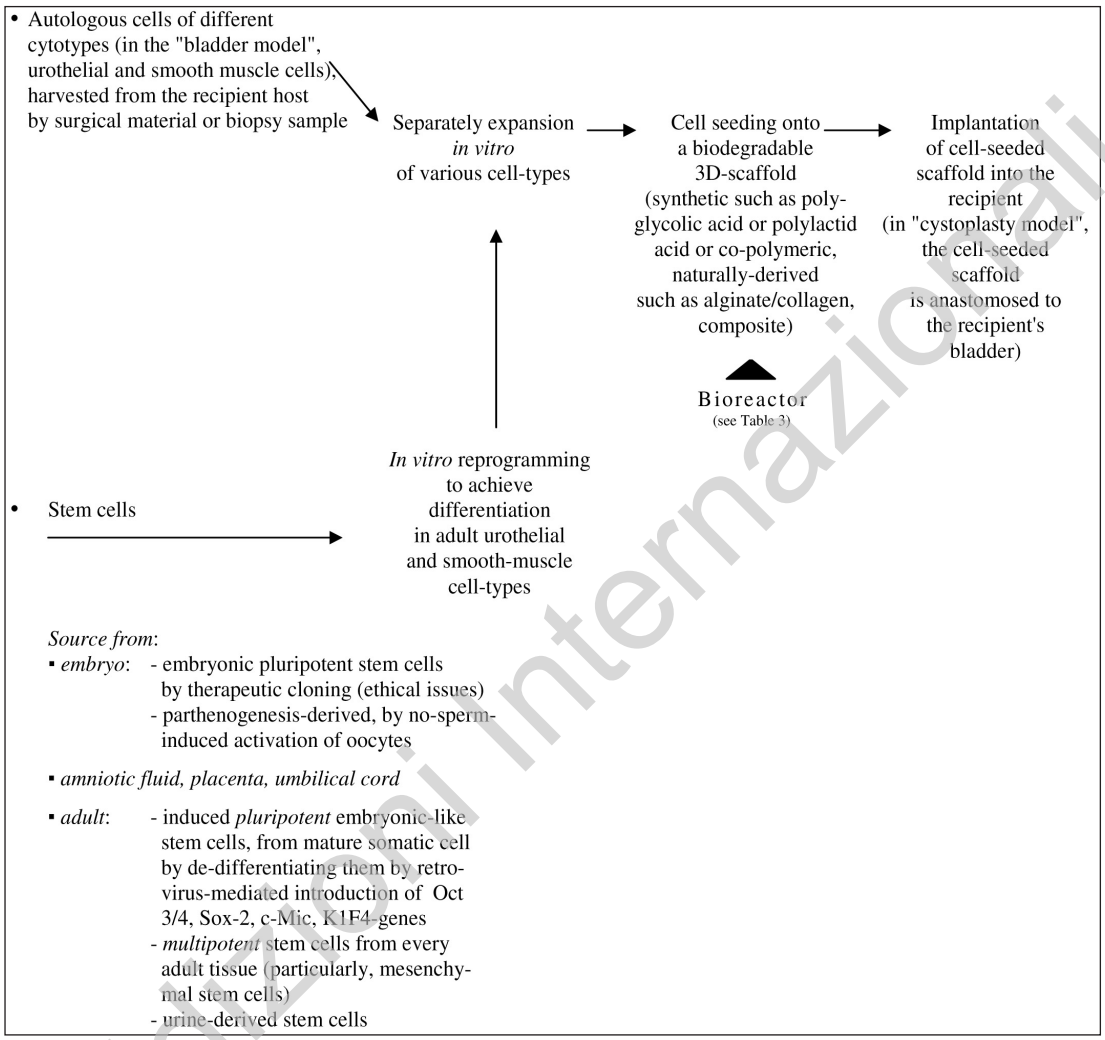
plications include chronic inflammatory response of intestinal prosthesis to urine with increased mucus production, stone formation, incomplete reservoir emptying, reasorption of some drugs (antibiotics, antitumoral chemotherapeuticals, etc) and their active metabolites with following increased related-toxicity towards the excretory target organs such as liver and kidney (12-15).

In order to prevent such complications and considering that the resort to an intestinal segment, in bladder reconstructive surgery, must be avoided in different conditions – short gut syndrome, chronic inflammatory disease, outcomes of abdominal irradiation – research has been led, in ancient times, to utilizing alloplastic materials (polytetrafluoroethylene, silicon rubber, polyuretane, polyvinil/polyester sponge) which have been discarted because of material-related absolutely negative outcomes, and, in the last decade of past century, to pioneeringly engineering an artificial bladder tissue able to quite mimic both the barrier/sensory transducer urothelial properties and the dynamic smooth muscle aptitudes (1, 3, 16-18).

The autologous urothelial and smooth muscle cell-based tissue engineering technology (Table 2) successfully carried out, at first, in different animal models, has achieved nine years ago, the expected good *clinical validation* in patients requiring the augmentation cystoplasty because of end-stage, myelomeningocele-related, poorly compliant neuropatic bladder; even on the recent follow-up, such cystoplasty displayed a normally organized graft wall architecture, consisting of urothelial, suburothelial connective and smooth muscle layers, at the same time showing both the urodynamic and renal function normal parameters and no systemic metabolic complications (19, 20).

Nevertheless, the use of autologous bladder cells, in patients with invasive bladder malignancy, must be warily approached because the whole vesical wall, though macroscopically normal, could contain some scattered areas of carcinoma *in situ* or urothelial chromosomal aberrations. Moreover other limitations such as complications potentially associated with invasive tissue biopsies and, on the other hand, low *in vitro* proliferative ability of adult mature cells, have suggested the resort to other cell sources, among which, especially, the pluri- and multipotent *stem cells*, that, besides their self-renewal, are capable of differentiating, under adequate conditions, in various tissue-specific cell lineages (Table 1) (21-23). However, in patients with bladder carcinoma, the implant of an autologous bladder multipotent stem cell-derived tissue engineered reservoir could induce a cancer transmission, hence an alternative option may result from the use of every mature tissue-derived iPSC, so avoiding the ethical limitations associated with the use of therapeutic cloning-derived embryonic stem cells (Table 1) (22, 24, 25). Moreover, the ability of *bone-marrow-derived mesenchymal stem cells* ($BMSC_S$) to simultaneously differentiate into urothelial and smooth muscle cells may have useful clinical application, in bladder tissue-engineering, for patients with bladder cancer or extrophy (22, 26, 27).

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



(mod. from Alberti C. Eur Rev Med Pharmacol Sci 2007; 11: 257-264).

Nevertheless, the low quantity and poor proliferation of certain populations of stem cells, such as $BMSC_S$ especially in elderly patients, and considering that invasive procedures are often required to achieve the amount of stem cells for the regenerative medicine, it has been suggested to resort to dedifferentiated fat (DFAT) cells – *preadipocyte cells* – that may be obtained from mature adipocytes of subcutaneous adipose tissue and can differentiate into smooth muscle phenotype *in vitro* via activation of TGF-ß signaling pathway, it representing an attractive strategy for the bladder tissue engineering (28, 29).

Even *urine-derived stem cells* (USC) can originate, under adequate conditions, both urothelial and smooth muscle cells, so allowing the fabrication of *tissue-engineered conduit*, seeding them onto a bacterial cellulose polymer scaffold (30).

Tissue engineered trachea

Beyond the experimental studies in animal models, 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, seven years ago, to repair an airway defect resulting, at the anastomotic site, from carinal pneumonectomy (31).

Three years ago, the world's first clinical successful *replacement of whole tissue engineered airway*

TABLE 3 - TRANSPLANTATION OF THE BIOENGINEERED DONOR ORGAN ("AIRWAY MODEL").

Human donor organ (in "airway model", donor tracheal segment)	→ Removal of all donor cells and MHC-antigens by detergent-enzymatic (DNase) treatment, to obtain *an organ-shaped-connective tissue scaffold* (in "airway model", tracheal extracellular matrix)	→ Colonization of such donor scaffold by recipient cells (in "airway model, ciliated respiratory epithelial cells and mesenchymal stem-cell-derived chondrocytes)	→ Graft then used to replace the recipient's diseased organ (in this model, left main bronchus)

▲

Bioreactor

Automatized dynamic cell culture system, that provides *in vitro* biochemo-physical and biomechanical dynamic conditions, mimicking the *in vivo* environment, to allow not only the growth of cells seeded onto the scaffold with a correct histotecnonics, but also the development of native tissue-like physiological properties

(mod. from Alberti C. G Chir 2009; 30: 514-519)

has been performed in 30-year-old woman with end stage post-tuberculosis malacia-induced left main bronchus collapse, by implantation of autologous stem cell-based tissue engineered windpipe, its scaffold resulting from a decellularized human donor trachea (Table 3). The dead donor trachea was, indeed, decellularized by a detergent enzymatic technique, to achieve the complete removal of donor MHC antigens without denaturating the underlying collagenous matrix structure which, then, was seeded *in vitro* with recipient patient mesenchymal stem cell-derived cartilage-like cells and autologous respiratory epithelial cells, thus obtaining a living tissue engineered airway segment to replace the recipient's diseased bronchus, without the risk of rejection (32).

Nevertheless, a drawback of a lengthy time (almost three months) to produce the graft – obviously it representing an unfeasible condition for patients in an urgent need of airway transplantation – drove to speed up the human donor organ decellularization process (about three weeks, more compatible with clinical needs) to obtain an acellular human windpipe scaffold, yet it retaining hierarchical structures and mechanical properties of the native trachea together with preservation of its angiogenic factors (33, 34).

The ongoing research in airway alternative regenerative approach is moving towards *in situ* organ regeneration by resorting to a bionic tissue engineered transplantation method (35).

Quite recently (June 9, 2011), an implant of artificial trachea, made up of autologous mesenchymal stem cells seeded onto synthetic polymer-nanoparticle scaffold, has been successfully performed by Macchiarini's group (Karolinska University Hospital, Stockholm) in 36-year-old trachea cancer patient.

Tissue engineered small intestine

The massive resection of small intestine because of either chronic inflammatory or malignant bowel disease, can induce a severe short-gut syndrome needing the parenteral nutrition providing patients with an adequate supply of nutrients and calories but exposing them to the risk of central venous catheter complications and parenteral nutrition-related liver disease.

The development of tissue engineering technologies could lead to their applications in the bowel replacement surgery.

In animal (rodent) models, the intraomentally implantation of autologous neonatal small intestine *organoid units*, attached to a biodegradable polymer scaffold and preliminary cultured in functional perfusion bioreactor, induced the production of the rudimentary epithelium-lined cystic structures, whose patches, after anastomosis to the native intestine, developed into the mature mucosa with normal crypts and villi. Subsequently, the application of such technology to a short bowel syn-

drome rodent model induced a successful intestinal segment regeneration with improvement of the recipient rodent's health (36, 37). In a recent pig model study – intraperitoneally implantation of autologous neonatal intestinal organoids units attached onto biodegradable scaffolds –, the architecture of the tissue engineered small intestine appears to replicate that of native bowel, showing an intestinal mucosa with differentiated epithelial cell types, together with intestinal stem cells at the base of the crypts, upon an innervated muscolaris mucosa (38). Most published studies report a prevailing use of PLA/PGA (polylactic acid/polyglycolic acid) copolimer as a scaffold for autologous cellular component, while seldom a new nanocomposite of polyhedral oligomeric silsesquioxane/poly (caprolactone-urea) urethane (POSS/PCL) has been exploited as a material to produce porous shape structure, in various pore sizes, to allow an appropriate intestinal epithelial cell proliferation (39).

These successful experimental outcomes are now encouraging the research interests in the therapeutic potential of a human intestine tissue engineering, given that an autologous tissue engineered small intestine could resolve severe conditions of short-gut syndrome at the same time avoiding the intestinal homotransplant-related critical problems among which the organ donor availability and the immunosuppressive treatment-associated complications (36-41).

Unfortunately, some important obstacles seem to stand in the way of the *human intestine tissue engineering* such as, firstly, the difficulties to find a source of autologous intestinal epithelial cells given the obvious unfeasibility, in humans, of the technical procedure followed in animal models (use, as above-mentioned, of intestinal organoids obtained from neonatal animals because older animal-derived intestinal organoids fail to produce epithelial cell lined-cystic structures) (36). Hence, the fulfillment of the human intestine tissue engineering could result from either using autologous intestinal stem cells, by preliminary identifying their specific markers, to produce intestinal organoids, or resorting to $iPSC_S$. The differentiation of human $iPSC_S$ into intestinal tissue *in vitro* using a precise sequence of growth factor supplies to mimic the intestinal embryogenesis, has allowed the formation of 3D-intestinal organoids with a polarized columnar epithelium showing villus-like structures together with crypt-like proliferative areas provided with intestinal stem cells and containing differentiated functional enterocytes, goblet-, neuroendocrine-, and Paneth-cells (42)..

Conclusions

The ongoing evolution in biomaterial sciences and, particularly, the breakthroughs of the bioengineering technologies by using autologous cells seeded onto a scaffold obtained from either donor organ ready made natural matrix structure or *de novo* biodegradable material-constituted product, allow today to make available immunologically personalized tissue-engineered prostheses, so avoiding the risk of rejection and, therefore, the resort to long-life immunosuppressive therapy. *Synthetic scaffolds* have the advantage that the construct may be tailored to a somatic organ features of a particular subject, moreover without that their availability might be affected by a limited dead donor pool, whereas the *decellularized scaffolds*, with their own vascular networks, are better fulfilling both anatomic and functional properties of the organ to be replaced (2, 19, 24, 32, 42-44). With regard to synthetic scaffold, the development of a bilayered scaffolding method, that allows the endothelial cell adhesion on the lumen (inner layer) and smooth muscle cell infiltration into the outer layer, may improve the blood vessel tissue engineering (45).

The progress in the understanding of the *stem cell* isolation, culture and differentiate lineage programming enhances the technical chances for organ replacement surgery.

The tissue engineering of hollow organs such as bladder, trachea and small intestine, appears to be an intriguing model to investigate it-related translational problems – from bench to bedside – about which gradually discovering both biological and technical suitable solutions. Indeed, on the basis of these conceptual outlines, also the vaginal reconstruction, in human patients, by "off-the-shelf" neovagina, made up through tissue engineering technology by resort to either autologous mature vaginal cells, if it is possible, or autologous iPSC, seems to be getting close (46).

As far as the *bladder replacement in surgical oncology* is concerned, there would be more advantages to have the availability, after total cystectomy, of a whole bladder prosthesis with a trigone-shaped base to be anastomosed with the native ureters and urethra (47).

Organ bioengineering technology by the development of a transplantable recellularized organ

graft, by using, as natural vascularized scaffold, a decellularized dead donor organ matrix, could usefully applied to generate transplantable whole human *solid modular organs-needing vascular network*, such as liver, kidney, heart, small intestine (4, 6-8, 43). In this regard, tissue engineering may also benefit from directed tissue-assembly, by the 3D-micromasonry of cell-laden microgel subunits with high resolution through both microscale self-assembly and microfluidics (48, 49).

What's more, different human whole organ engineering models, together with the development of bioreactor technology, allow to study, on the bench, the drug pharmacokinetics and pharmacodynamics, as an alternative to the animal experiments (4).

References

1. Atala A, Vacanti JP, Peters CA, Retik AB, Mandell J, Freeman MR. Formation of urothelial structure *in vivo* from dissociated cells attached to biodegradable polymer scaffold *in vitro*. J Urol 1992; 148: 658-662.
2. Birchall MA. Cell- and tissue-engineered organ replacements. Br J Surg 2009; 96: 565-566
3. Atala A. Engineering organs. Curr Opin Biotechnol 2009; 20: 575-592.
4. Schanz J, Pusch J, Hansmann J, Walles H. Vascularized human tissue models: a new approach for the refinement of biochemical research. J Biotechnol 2010; 148: 56-63.
5. Preti RA. Bringing safe and effective cell therapies to the bedside. Nature Biotechnol 2005; 23: 801-804.
6. Orlando G, Baptista P, Birchall M, De Coppi P, Farney A, Guimaraes-Souza NK, Opara E, Rogers J, Seliktar D, Shapira-Schweitzer K, Stratta RJ, Atala A, Wood KJ, Soker S. Regenerative medicine as applied to solid organ transplantation: current status and future challenges. Transpl Int 2011; 24: 223-232.
7. Uygam BE, Soto-Gutierrez A, Yagi H, Izamis ML, Guzzardi MA, Shulman C, Milwid J, Kobayashi N, Tilles A, Berthiaume F, Herti M, Nahmias Y, Yamush ML, Uygum K. Organ reengineering through development of a transplantable recellularized liver graft using decellularized liver matrix. Nat Med 2010; 16: 814-820.
8. Baptista PM, Orlando G, Mirmalek-Sani SH, Siddiqui M, Atala A, Soker S. Whole organ decellularization: a tool for biscaffold fabrication and organ bioengineering. Conf Proc IEEE Eng Med Biol Soc 2009; 2009: 6526-6529.
9. Chen HC, Hu VC. Bioreactors in tissue engineering. Biotechnol Lett 2006; 28: 1415-1423.
10. Arrigoni C, Chittò A, Mantero S, Remuzzi A. Rotating versus perfusion bioreactor for the culture of engineered vascular constructs based on hyaluronic acid. Biotechnol Bioeng 2008; 100: 988-997.
11. Berry JL, Steen JA, Koudi Williams J, Jordan JE, Atala A, Yoo JJ. Bioreactors for development of tissue engineered heart valves. Ann Biomed Eng 2010; 38: 3272-3279.
12. Alberti C. From the intestinal neobladder to the bioartificial bladder: some biological implications. Minerva Urol Nephrol 2000; 52: 219-222.
13. Mc Dougal WS. Metabolic complications of urinary intestinal diversion. J Urol 1992; 147: 1199-1208.
14. Gilbert SM, Hensle T. Metabolic consequences and long-term complications of enterocystoplasty in children: a review. J Urol 2005; 173: 1080-1086.
15. Alberti C. Metabolic and histological complications in ileal urinary diversion. Challenges of tissue engineering technology to avoid them. Eur Rev Med Pharmacol Sci 2007; 11: 257-264.
16. Oberpenning F, Meng J, Joo JJ, Atala A. *De novo* reconstitution of a functional urinary bladder by tissue engineering. Nat Biotechnol 1999; 17: 149-155.
17. Atala A. Tissue engineering for the replacement of organ function in the genitourinary system. Am J Transplant 2004; 4 (suppl 6): 58-73.
18. Cilento BG, Freeman MR, Schneck FX, Retik AB, Atala A. Phenotypic and cytogenetic characterization of human bladder urothelia expanded *in vitro*. J Urol 1994; 152: 665-670.
19. Atala A, Bauer SB, Soker S, Yoo J, Retik S. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 2006; 367: 1241-1246.
20. Atala A, Joo JJ. Methods in tissue engineering. Methods 2009; 47: 79-80.
21. Cross WR, Thomas DFM, Southgate J. Tissue-engineering and stem cell research in urology. BJU Int 2003; 92: 165-171.
22. Yamamoto H, Shah S, Masters JR, Popert RJ, Dasgupta P. Stem cells in regenerative urology of the bladder. BJU Int 2009; 104: 1183-1186.
23. Becker Ch, Jakse G. Stem cells for regeneration or urological structures. Eur Urol 2007; 51: 1217-1228.
24. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448: 313-317.
25. Amabile G, Meissner A. Induced pluripotent stem cells: current problems and potential for regenerative medicine. Trends Molecular Med 2009; 15: 59-68.

26. Tian H, Bharadwaj S, Liu Y, Ma PX, Atala A, Zhang Y. Differentiation of human bone marrow mesenchymal stem cells into bladder cells: potential for urological tissue engineering. Tissue Eng Part A 2010; 16: 1769-1779.
27. Tian H, Bharadwaj S, Liu Y, Ma PX, Atala A, Zhang Y. Myogenic differentiation of human bone marrow mesenchymal stem cells on a 3D-nano fibrous scaffold for bladder tissue engineering. Biomaterials 2010; 31: 870-877.
28. Sakuma T, Matsumoto T, Kano K, Fukuda N, Obinata D, Yamaguchi K, Yoshida T, Takahashi S, Mugishima H. Mature adipocyte-derived dedifferentiated fat cells can differentiate into smooth muscle-like cells and contribute to bladder tissue regeneration. J Urol 2009; 182: 355-365.
29. Yagi K, Kondo D, Okazaki Y, Kano K. A novel preadipocyte cell line established from mouse adult mature adipocytes. Biochem Biophys Res Commun 2004; 321: 967-977.
30. Bodin A, Bharadwaj S, Wu S, Gatenholm P, Atala A, Zhang Y. Tissue engineered conduit using urine-derived stem cells seeded on bacterial cellulose polymer in urinary reconstruction and diversion. Biomaterials 2010; 31: 8889-8901.
31. Macchiarini P, Walles Th, Biancosino Ch, Mertshing H. First human transplantation of a bioengineering airway tissue. J Thorac Cardiovascular Surg 2004; 128: 638-641.
32. Macchiarini P, Jungebluth P, Go T, Asnaghi MA, Ross LE, Cogan TA, Dodson A, Martorell J, Bellini S, Panigotto PP, Dickinson SC, Hollander AP, Mantero S, Conconi MT, Birchall MA. Clinical transplantation of a tissue-engineered airway. Lancet 2008; 372: 2023-2030.
33. Baiguera S, Jungebluth P, Burns A, Mavilla C, Haag J, De Coppi P, Macchiarini P. Tissue engineered human trachea for *in vivo* implantation. Biomaterials 2010; 31: 8931-8938.
34. Kolathur M, Baiguera S, Macchiarini P. Transplanting tissue-engineered tracheal replacement from bench to bedside. Cell Mol Life Sci 2010; 67: 4185-4196.
35. Bader A, Macchiarini P. Moving towards *in situ* tracheal regeneration: the bionic tissue engineered transplantation approach. J Cell Mol Med 2010; 14: 1889.
36. Dunn JCY. Is the tissue-engineered intestine clinically viable? Nat Clin Pract Gastroenterol Hepatol 2008; 5: 366-367.
37. Kim St, Penkala R, Abrahimi P. A perfusion bioreactor for intestinal tissue engineering. JSR 2007; 142: 327-33.1
38. Sala FG, Kunisaki SM, Ochoa ER, Vacanti J, Grikscheit TC. Tissue-engineered small intestine and stomach form from autologous tissue in preclinical large animal model. J Surg Res 2009; 156: 205-212.
39. Gupta A, Vara DS, Punshon G, Sales KM, Winslet MC, Selfaliam AM. In vitro small intestinal epithelial cell growth on a nanocomposite polycaprolactone scaffold. Biotechnol Appl Biochem 2009; 54: 221-229.
40. Pahari MP, Brown ML, Elias G, Nseir H, Banner B, Rastellini C, Cicalese L. Development of a bioartificial new intestinal segment using an acellular matrix scaffold. Gut 2007; 56: 885-886.
41. Choi RS, Vacanti JP. Preliminary studies of tissue-engineered intestine using isolated epithelial organoid units on tubular synthetic biodegradable scaffolds. Transplant Proc 1997; 15: 29848-29851.
42. Spence JR, Mayhew CN, Rankin SA, Kuhar MF, Vallance JE, Tolle K, Hoskins EE, Kalinichenko VV, Wells SI, Zom AM, Shroyer NF, Wells JM. Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. Nature 2011; 470: 105-109.
43. Saxena AK. Tissue engineering and regenerative medicine research perspectives for pediatric surgery. Ped Surg Int 2010; 26: 557-573.
44. Alberti C. Tissue engineering technologies: just a quick note about transplantation of bioengineered donor trachea and augmentation cystoplasty by *de novo* engineered bladder tissue. G Chir 2009; 30: 514-519.
45. Ju YM, Choi JS, Atala A, Joo JJ, Lee SJ. Bilayered scaffold for engineering cellularized blood vessels. Biomaterials 2010; 31: 4313-4321.
46. Dorin RP, Atala A, Defilippo RE. Bioengineering a vaginal replacement using a small biopsy of autologous tissue. Semin Reprod Med 2011; 29: 38-44.
47. Alberti C. Bladder regeneration by tissue engineering (Atala's reply). BJU Int 2002; 89: 972-973.
48. Khademhosseini A, Langer R, Borenstein J, Vacanti JP. Microscale technologies for tissue engineering and biology. Proc Natl Acad Sci USA 2006; 21: 2480-2487.
49. Khademhosseini A, Fernandez JG. Micro-masonry: construction of 3D structures by microscale self-assembly. Adv Mater 2010; 22: 1-4.
50. Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Närvä E et al. Copy number variation and selection during reprogramming to pluripotency. Nature 2011; 471: 58-62.
51. Szabo E, Rampalli S, Risueño RM, Schnerch A, Mitchell R, Fiebig-Comin A, Levadoux-Martin M, Bhalia M. Direct conversion of human fibroblasts to multilineage blood cell progenitors. Nature 2010; 468: 521-526.