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review

Whyever bladder tissue engineering clinical applications still remain unusual even though many intriguing technological advances have been reached?

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SUMMARY: **Whyever bladder tissue engineering clinical applications still remain unusual even though many intriguing technological advances have been reached?**

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To prevent problematic outcomes of bowel-based bladder reconstructive surgery, such as prosthetic tumors and systemic metabolic complications, research works, to either regenerate and strengthen failing organ or build organ replacement biosubstitute, have been turned, from 90s of the last century, to both regenerative medicine and tissue engineering. Various types of acellular matrices, naturally-derived materials, synthetic polymers have been used for either "unseeded" (cell free) or autologous "cell seeded" tissue engineering scaffolds. Different categories of cell sources – from autologous differentiated urothelial and smooth muscle cells to natural or laboratory procedure-derived stem cells – have been taken into consideration to reach the construction of suitable "cell seeded" templates. Current clinically validated bladder tissue engineering approaches essentially consist of augmentation cystoplasty in patients suffering from poorly compliant neuropathic bladder. No clinical applications of wholly tissue engineered neobladder have been carried out to radical-reconstructive surgical treatment of bladder malignancies or chronic inflammation-due vesical coarctation. Reliable reasons why bladder tissue engineering clinical applications so far remain unusual, particularly imply the risk of graft ischemia, hence its both fibrous contraction and even worse perforation. Therefore, the achievement of graft vascular network (vasculogenesis) could allow, together with the promotion of host surrounding vessel sprouting (angiogenesis), an effective graft blood supply, so avoiding the ischemia-related serious complications.

KEY WORDS: Tissue engineering - Reconstructive surgery - Augmentation cystoplasty - Orthotopic neobladder - Neurogenic bladder - Oncology.

Introduction

About the bladder reconstructive surgery – from bladder augmentation cystoplasty to orthotopic whole neobladder – the *use of autologous bowel segments*, though with the onset sometimes of both prosthetic malignancies and systemic metabolic complications, still remains the gold standard since no better alternative has been proved to be wholly reliable.

With regard to such *pathomorphosic and malignant complications*, clinical and animal model histologic examinations on prosthetic intestinal segments may at times show a sequential pathway from chronic urine exposure-related inflammatory conditions to malignant transformation, given that phlogogenic cells can also overexpress cancerogenic cytokines. Besides the adenocarcinoma, other tumors – including polypoid adenoma, signet ring carcinoma, transitional cell carcinoma, sarcoma, lymphoma and carcinoid – may affect the intestinal urinary diversion, particularly that colonic rather than the ileal one. Preternatural histotectonic connections between ureteral transitional epithelium and paranastomotic intestinal mucosa most likely explain, through altered cell growth signalling between two dissimilar cell compartments, the prevailing appearance of malignant changes just at the uretero-intestinal suture line (1-4).

Systemic metabolic imbalances of intestinal urinary diversion arise from both the chronic exposure of bowel to urine – response to excess of urinary NH_4, H^+, Cl^- absorption with developing iperchloremic acidosis, whence hypokalemia, bone demineralization with resulting hyperphosphatemia/hyperphosphaturia and phosphate urinary stone formation (that's made easier given the mucus overexpression) – and, otherwise, the reconstructive measure-due removal of ileal segment with following chologenic diarrhoea/steatorrhoea, hype-

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roxaluria and oxalate urolithiasis, hypocalcemia and hypomagnesemia, vit. B_{12} deficiency. Moreover, because of osmotic gradient, the ileal reservoir may lose quite a lot of water into the lumen while, on the other direction, some drugs (antibiotics, methotrexate, etc.) or their active metabolites may be reabsorbed, thus it increasing toxicity towards the liver, as its pivotal role in drug metabolism, and the kidney as excretory target organ (5-8).

Just to prevent such problematic disadvantages and, in addition, considering that the use of bowel in bladder reconstructive surgery must be avoided in some pathological conditions – such as chronic inflammatory disease, short gut syndrome, abdominal/pelvic external been radiation therapy – intriguing studies from the 90s of the last century, have been directed to more and more develop the *tissue engineering technologies* for *de novo* urinary bladder substitute construction, including bladder both augmentation cystoplasty and orthotopic replacement.

Nevertheless, as it will be down here set out, also the resort to tissue engineering measures may dangerously imply some disagreeable – particularly graft limited vascularity-related – outcomes (9-13).

Regenerative medicine and tissue engineering research developments

Regenerative medicine and tissue engineering technological developments, during the last two decades, made more and more feasible the creation of functional bio-structures to either regenerate and strengthen failing organs or build organ replacement bio-substitutes (1, 5, 14).

The ideal artificial bladder should show native urinary bladder-like properties such as particularly the ability to effectively store urine at low pressure and, otherwise, allow a voluntary voiding with minimal prosthesis-ureter reflux (2).

Tissue engineering scaffold materials include either acellular tissue matrices or naturally-derived extra-cellular matrix (ECM) components or also several biodegradable synthetic polymers (Table 1).

For a long time now, it has been reported that acellular matrices – such as bladder acellular matrix (BAM) and small intestinal submucosa (SIS) – are able to sustain the proliferation of urothelium and smooth muscle cells arising from adjacent normal tissue together with the blood vessel and nerve regeneration (15-17). Current developments in building BAM scaffolds aim to facilitate the interactions between such matrix and surrounding tissue cells so that allow the output of cell-seeded grafts that might be used as "off the shelf" replacement material for augmentation bladder cystoplasty (17). SIS-based bladder tissue regenerative medicine allow the whole reconstruction of three normal bladder like-layers (mucosa/submucosa, smooth muscle detrusor and serosa) together with their vascular network (5, 18, 19). What's more, regrowth of

TABLE 1 - DIFFERENT CLASSES OF BIOMATERIALS MAINLY USED TO BUILD BLADDER TISSUE ENGINEERING SCAFFOLDS.

▪ Acellular tissue matrices by removing all tissue cell components	-	bladder acellular matrix, BAM
	-	small intestinal submucosa, SIS
▪ Naturally-derived materials as extracellular matrix polymer components	-	protein-based, as collagen, elastin, laminin, fibronectin
	-	carbohydrate-based, as alginate, hyaluronic acid, agarose, chitosan
Both such classes of biomaterials are naturally endowed with bio-properties that allow to better mimic native tissue extracellular matrix (ECM), although their use might result difficult given that they are limited in supply and show poor mechanical features (particularly, lack of micro/nanoscale structuration) besides may cause immune responses.		
▪ Synthetic polymers		polyglicolic acid, PGA polylactic acid, PLA polylactic-co-glycolic acid, PLGA polycaprolactone, PLC polycaprolactone-co-lactic acid, PCL-PLA polycarbonate urethane-urea, PCUU
Synthetic polymer-based scaffolds may be built on a large scale with suitable features of micro-nanostructure, strength and degradation process.		
▪ Composite scaffolds: consisting of both a ECM component, such as collagen, and a synthetic polymer, are particularly used for hollow genitourinary organ building.		

smooth muscle layer using SIS appears to promote normal contractile activity given, indeed, the native bladder tissue-like expression of purinergic, muscarinic, β-adrenergic receptors (20).

Current bladder tissue engineering modalities include both *unseeded* (cell free matrix) and *seeded* (cell matrix) scaffold strategies, the first directed to promote a natural *in vivo* process of wall regeneration with arising urothelial and smooth muscle cells from surrounding native tissue (guided regenerative medicine) while the second, instead, by seeding recipient autologous urothelial/smooth muscle cells or stem cells onto the scaffold, is directed to build *in vitro* replacement functional tissues or organs (true tissue engineering) (21-23). Suitably cell-seeded scaffolds should today allow to obtain tissue engineered constructs endowed with functional native bladder tissue-like properties, among which the urothelium-due both the impermeability – as barrier towards urinary solute absorption so that avoid the metabolic imbalances – and bladder filling pressure sensory transducer function to modulate tissue engineered organ dynamics, together with smooth muscle cell phenotype-related contractility/relaxation activity (24, 25).

Synthetic polymers (Table 1) for cell-seeded 3D scaffold-based bladder tissue engineering, besides to be endowed with essential biocompatibility properties – such as to be biodegradable and bioresorbable, anti-bacterio-/mycostatic, nonphlogogenic without inducing foreign-body tissue reactions, nonimmunogenic and noncancerogenic – must be able to adequately support seeded cells kinetic peculiarities (adhesion, proliferation, differentiation, migration) particularly due to interactions of specific soluble growth factors with transmembrane cell integrin receptors (25-28).

In this regard, novel so-called "small biomaterials" – showing significant conformational changes in response to small microenvironmental physico-chemical variations – have been devised, particularly including materials incorporating specific protein domains, among which RDG (arginine-glycine-aspartic acid compound) as molecular domain of fibronectin that is able to interact with α5β1 and αVβ3 integrin-cell surface receptors (26-31).

Also intriguing advances in the field of *nanotechnologies,* concerning the treatment of various materials at micro/nano level (from 100 μm to 10 nm), have led, over two last decades, to improve the cell/scaffold interactive connections. By mimicking the nanoscale architecture of native bladder tissue, the synthetic polymer-based scaffolds with nanostructured surface make cell adhesion/growth easier than that of bladder tissue building conventional modalities, as nanoscale polymer structures showing the same nano-size of natural tissue constitutive cell proteins, especially those of the cell surface receptors, so reaching the advantage of "directly speaking the language of cells" (32-35).

Micro/nanometer scale-based polymeric structures may be achieved by several techniques, among which thermodynamic procedure of polymeric solutions (from gas foaming to freeze drying), electrospinning as a generation modality of polymeric nanofibers by high electropotentials, and nano-optical photolithography to build polymer hydrogel-based micro/nanostructured scaffolds (33, 34). Particularly, new electrospinning built hybrid poly-ε-caprolactone (PCL) and poly-L-lactic acid (PLLA) nano-scale scaffolds allow to clearly validate the biocompatibility and effectiveness of such polymers in the bladder tissue engineering (36).

Different bladder tissue engineering nanotechnology approaches – including either nanomaterials to coat conventionally made scaffold surfaces or *de novo* electrospun nanofiber-built scaffolds – show many structural advantages, in comparison with non-nanostructured constructs, including the lower calcium oxalate stone formation (32, 37).

The integration of synthetic polymers with ECM native components – such as collagen, fibronectin, laminin, elastine – may lead to obtain significant improvements of both the various cell type binding to scaffolds and the growth of different functional engineered tissues (27, 30). What has been recently proved by comparison of nanometer-size fibronectin/gelatin layer-coated electrospun fibrous poly(carbonate urethane) urea-based scaffolds with the uncoated ones (38).

As pivotal tools to properly develop tissue engineering organs, different *bioreactors* can provide, just regarding bladder mechanical properties (wall elasticity, compliance pertinent various filling pressure, etc), suitable mechanical stimulations in cell culture chambres, thus favourably affecting both urothelial and smooth muscle cell growth together with the endothelial cell layer development in blood vessels (39, 40).

Advanced molecular and imaging technologies, such respectively next generation sequencing and magnetic resonance, may be used for monitoring regeneration processes (41).

Though out of the true synthetic biomaterials field,"silk fibroin"made scaffolds, coated with ECM proteins, have been timely used, in animal model bladder tissue engineering, as templates for urothelial/smooth muscle cell seeding procedure, thus achieving a construct endowed with effective structural and functional proper such as particularly biodegradability, plasticity, compliance (42, 43). In this regard, recent animal model studies have shown that the stretched electrospun silk fibroin matrix implantation can strongly promote bladder tissue regeneration with proper structure/function features, in comparison with BAM (44).

Essentials of various cell type source for cell-seeded scaffolds

The bladder-shaped scaffold seeded with autologous differentiated both urothelial and smooth muscle cells, previously harvested from recipient host by surgical material or biopsy sample and expanded in culture, still remains the ideal option for bladder tissue engineering, given that has the advantage of avoiding the tissue rejection and immunosuppressive treatment-related adverse effects. Towards the opinion of poor sample healthy cell availability given the frequent large host bladder tissue disease, it has been verified that the native tissue-specific progenitor unipotent cells – able to self-renewal and appropriate differentiation – may remain normal even in occasion of diseased tissue (e.g., neuropathic bladder) (5).

It has been recently shown that *in vitro* bladder graft preparation by type-1 collagen substrate seeded with bladder mesenchymal cells, can even generate, when exposed to urine, a physiologically pseudostratified urothelium. Moreover, promising chances of cell-seeded bladder engineering may be achieved by the resort to pluripotent or multipotent *stem cells* (Table 2), capable of self-renewal together with properly guided differentiation in tissue-specific cell lineages, such as particularly both urothelial and smooth muscle cells (13, 45-47).

Clinical validation of bladder tissue engineering

Current clinically validated *de novo* bladder tissue engineering approaches essentially consist of successful augmentation cystoplasty, in patients suffering from neurogenic/neuropathic bladder, by either using host autologous mature urothelial/smooth muscle cells seeded onto PGA-collagen composite 3D-scaffold – post-implantation wisely covered with omental drap (40, 48) – or resorting to SIS as acellular tissue matrix-based scaffold to regenerate a functional bladder tissue (49). Satisfactory outcomes of such above procedures have been properly assessed by serial urodynamics, diagnostic imaging (US, Xray, RM) and blood/urine laboratory tests.

TABLE 2 - HUMAN STEM CELLS: TYPOLOGY-RELATED SPECIFIC FEATURES.

Naturally-derived stem cells	
From embryo	• Totipotent stem cells: identifying with the zigote and pertinent to earliest embryogenic phase up to morula, are able to develop into all three germ cell layers together with also generating extraembryonal structures such as placenta. • Pluripotent stem cells: from the blastocyst to the end of embryogenic phase, are able to generate all three germ cell layers whereas risultant ineffective to generate extraembryonal structures.
From foetus	• Multipotent stem cells: as derived from different foetus tissues, they give rise to limited organ-pertaining proper tissue-cells output. In this regard, also the amniotic fluid stem cells, as derived from a developing foetus, are quite multipotent.
From adult	• Multipotent stem cells to a limited extent: as derived from different adult tissues – such as bone marrow, fat, skin, etc. – can generate limited mature cell-type lineages. Particularly, bone-marrow includes two different stem cell populations, whose the one properly "hematopoietic", able to produce all blood cells, while the other, so-called "stromal-mesenchymal",is effective to generate bone-, cartilage-, fat- and connective cells. • Unipotent stem cells: different stem cell types, placed in defined niches of each adult tissue, show only tissue cell-specific differentiative potentials.
Laboratory procedure-achieved stem cells	
• Resort to therapeutic cloning: mature somatic cell "nuclear transfer" to achieve production of autologous embryonic pluripotent stem cells. • Resort to genetic reprogramming: retrovirus-mediated transfection of transcription factors (Oct 3/4,Sox2,c-Myc,M1f4, so-called "Yamanaka factors"(45), or Oct4,Sox2,Nanog,Lin28 without introducing, thanks to Thompson group (46), c--Myc oncogene) in adult differentiated somatic cells, to induce generation of autologous pluripotent stem cells (iPSC).	

With reference to other clinical contributions, the bladder augmentation using an acellular biomatrix, as a pilot experience in exstrophic bladder patients, unfortunately failed to reach long-term effective outcomes, such as satisfactory bladder capacity and compliance together with urinary continence (50).

However, from recent literature reports it results that BAM could be effectively used as a cell-seeded graft to build "off the shelf " replacement materials for augmentation cystoplasty (17).

No clinical applications of wholly tissue engineered neobladder, including trigone/vesical neck, has been so far carried out to radical/reconstructive surgery for both bladder malignancies and chronic inflammation-induced vesical coarctation (51). The related surgical alternative measures should be restricted to tissue engineered urinary conduits that, though potentially directed to avoid the bowel-based urinary conduit complications meanwhile simplifying the Bricker's operation related surgical procedure, are unable to improve the quality of life, similarly they needing urinary diversion-linked external urine reservoir.

It follows that the auspicious foreseeable bladder tissue engineered construct could *in vivo* consist of either an orthotopic neobladder or, at least, a continent pouch-based cutaneous urinary diversion.

Reliable difficulties about the bladder tissue engineering clinical applications

Notwithstanding the great development of tissue engineering technologies, current clinical applications are unusual, many research challenges still remaining open (13).

Reliable reasons why bladder tissue engineering clinical applications so far are unusual, imply the significant risk of possible serious complications – particularly graft ischemia, hence its both fibrous contraction and even worse perforation – obviously due to limited post-implanted graft vascularity (12, 13).

Only the resort to both graft *de novo* blood vessel creation (vasculogenesis) and host existing vessel sprouting promotion by proper angiogenic factors (angiogenesis) may allow the effective vascular graft/host inosculation, thus preventing such disagreeable complications (12). It is resulting, indeed, from thorough studies in animal models, that a properly structured graft functional vascular network is essential, by suitable supply of oxygen and nutriens, for graft-host post-implant integration and tissue engineered survival (9). In this regard, recent studies show that the use of BAM allografts provided with VEGF (vascular endothelial growth factor) - and bFGF (basic fibroblast growth factor) loated PLGA (polylactic-co-glycolic acid) nanoparticles can rapidly restore graft vascularization meanwhile inhibiting contracture of regenerated bladder tissue in augmentation cystoplasties (10). What seems to be also reached by incorporation of trophic growth factors, among which VEGF and NGF, into cell seeded either BAM- or smart material-based scaffolds, leading to effective graft vascularization and innervation (11, 16, 52).

On the basis of what above reported, let's try to think that scaffolds consisting of acellular tissue matrices, with obviously ECM-natural polymer components, may structurally mimic pertinent bladder native tissue micro-architecture, better allowing the integration of graft reconstructed vascular network with surrounding host tissue vessels.

Conclusion

Although considerable advances have been made in the field of tissue engineering technologies, however, as far as the clinical applications are concerned, many challenges still remain to reach a significant consistent success.

Current research developments aim at deepening the studies on"smart scaffold"preparation, nanotechnology modalities, stem cell source biology, so that enhance the bladder tissue engineering feasibility (1, 5, 52, 53).

In this regard, particularly a quickly achievement of graft functional vascular network, to avoid ischemia-related serious complications, can provide timely solutions for a tissue engineering bladder successful clinical implementation (9, 12, 13).

Conflict of interest statement

The Author declares that the research was conducted in the absence of any potential conflict of interest.

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