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Outlines on nanotechnologies applied to bladder tissue engineering

C. ALBERTI

SUMMARY: **Outlines on nanotechnologies applied to bladder tissue engineering.**

C. ALBERTI

Tissue engineering technologies are more and more expanding as consequence of recent developments in the field of biomaterial science and nanotechnology research. An important issue in designing scaffold materials is that of recreating the ECM (extra-cellular matrix) functional features – particularly ECM-derived complex molecule signalling – to mimic its capability of directing cell-growth and neotissue morphogenesis. In this way the nanotechnology may offer intriguing chances, biomaterial nanoscale-based scaffold geometry behaving as nanomechanotransducer complex interacting with different cell nanosize proteins, especially with those of cell surface mechanoreceptors. To fabricate 3D-scaffold complex architectures, endowed with controlled geometry and functional properties, bottom-up approaches, based on molecular self-assembling of small building polymer units, are used, sometimes functionalizing them by incorporation of bioactive peptide sequences such as RDG (arginine – glycine – aspartic acid, a cell-integrin binding domain of fibronectin), whereas the top-down approaches are useful to fabricate micro/nanoscale structures, such as a microvasculature within an existing complex bioarchitecture. Synthetic polymer-based nanofibers, produced by electrospinning process, may be used to create fibrous scaffolds that can facilitate, given their nanostructured geometry and surface roughness, cell adhesion and growth. Also bladder tissue engineering may benefit by nanotechnology advances to achieve a better reliability of the bladder engineered tissue. Particularly, bladder smooth muscle cell adhesion to nanostructured polymeric surfaces is significantly enhanced in comparison with that to conventional biomaterials. Moreover nanostructured surfaces of bladder engineered tissue show a decreased calcium stone production. In a bladder tumor animal model, the dispersion of carbon nanofibers in a polymeric scaffold-based tissue engineered replacement neobladder, appears to inhibit a carcinogenic relapse in bladder prosthetic material. Facing the future, a full success of bladder tissue engineering will mainly depend on the progress of both biomaterial nanotechnologies and stem cell biology research.

RIASSUNTO: **Elementi sulle nanotecnologie applicate all'ingegneria tessutale della vescica.**

C. ALBERTI

La progressiva espansione delle tecnologie di ingegneria tissutale consegue a recenti sviluppi della ricerca nell'ambito dei biomateriali e delle nanotecnologie. Importante problema nella costruzione di materiali di supporto è quello di riprodurre le caratteristiche funzionali della matrice extra-cellulare (ECM) – particolarmente il complesso di segnali molecolari propri della ECM – al fine di imitarne le prerogative di guida nella proliferazione cellulare e morfogenesi tissutale. Sotto questo aspetto le nanotecnologie possono offrire interessanti prospettive poiché i supporti nanostrutturati si comportano come nano-meccanotrasduttori interagenti, con precisa corrispondenza geometrica, con le nanodimensioni delle varie proteine cellulari, specie con quelle dei meccanorecettori plasmalemmatici. Allo scopo di fabbricare complesse architetture di supporti tridimensionali con determinate caratteristiche geometriche e proprietà funzionali, si ricorre ad approcci "bottom-up", basati sullo autoassemblaggio di piccole unità costruttive polimeriche, talvolta funzionalizzate con sequenze bioattive di natura peptidica come RDG (arginina – glicina – ac. aspartico, costitutiva di un dominio della fibronectina, atto a legarsi alle integrine cellulari), mentre gli approcci top-down sono utili per costruire micro/nanostrutture, come la microvascolatura, all'interno di una preesistente bioarchitettura. Nanofibre polimeriche sintetiche, prodotte tramite "electrospinning" possono essere utilizzate per fabbricare supporti fibrosi che siano atti a facilitare, data la nano-configurazione e la ruvidezza della superficie, l'adesione e la proliferazione cellulare. Anche l'ingegneria tissutale della vescica può trarre vantaggi dagli sviluppi delle nanotecnologie al fine di raggiungere una miglior affidabilità del tessuto vescicale ingegnerizzato. In particolare, risulta significativamente migliorata l'adesione delle cellule muscolari vescicali a supporti polimerici nanostrutturati rispetto ai biomateriali convenzionali. Inoltre la superficie nanostrutturata di tessuto vescicale ingegnerizzato previene l'incrostazione calcica parietale e la litogenesi calcica. In animale da esperimento sottoposto a cistectomia totale per tumore vescicale sperimentalmente indotto, il ricorso a neovescica di rimpiazzo, realizzata con tessuto ingegnerizzato su supporto polimerico integrato con nanofibre di carbonio, evita la recidiva neoplastica nel materiale protesico. Quanto al futuro, il pieno successo della ingegneria tissutale vescicale dipenderà in gran parte dai progressi delle nanotecnologie applicate ai biomateriali e della ricerca sulla biologia delle cellule staminali.

KEY WORDS: Nanotechnology - Bottom-up, Top-down approaches - Hydrogels - Electrospinning - Stem cells.
Nanotecnologia - Approccio bottom-up e top-down - Hydrogel - Electrospinning - Cellule staminali.

L.D. of Surgical Semeiotics
University of Parma, Parma, Italy

For patients suffering from urinary bladder pathologies because of its either ontogenetic defects or acquired diseases (trauma, specific chronic inflammation, malignancy), there are unfortunately limited traditional organ replacement options that might allow a complete restoration of bladder functional features. Hence, the bladder tissue engineering technologies are more and more taken into consideration, nevertheless about them yet realizing several puzzling problems, particularly pertaining to bioscaffold design, choice of either stem cell- or differentiate cell-type, vascularization of regenerated tissue.

Intriguing discoveries in the field of nanotechnology have led, over two last decades, to a better understanding of cell-scaffold substrate interactions. Indeed, mimicking the nanoscale topography of native bladder tissue, nanostructured polymer biomaterial scaffold surfaces, as provided with appropriate nanoscale roughness, make cell adhesion/growth easier than in bladder tissue engineering conventional procedures (Table 1), by allowing the cell/scaffold interactions at the same size regime of constitutive cell-proteins, particularly of receptorial cell-surface proteins (1, 2). Thus, given such property, the nanotechnology applied to the bioengineering offers the advantage of «directly speaking the language of cells» (3).

Essentials on bio-nanotechnologies

The nanobiotechnology represents a broad interdisciplinary field concerning the manipulation of various materials at micro/nanometer level (from 100 *μm* to 10 *nm*), to obtain structures which might mimic the micro/nanoscale-based architecture of native tissues (4, 5).

The nanoparticle-modified scaffold surface – nanoparticles behaving as mechanotransducers interacting, at precise size geometry level, with different cell proteins, especially with those peculiar of cell surface mechanoreceptors – can make easier not only cell adhesion, -growth and -differentiation but also cell functions including cell molecule signalling, gene expression, hence protein synthesis. From that, it's possible to deduce that biomaterial nanostructure geometry (nanodots, nanopits, nanorods, nanopillars) significantly affects cell responses, maximum gene expression occurring when smallest size-based micropatterned substrates are used (4-8). Especially, nanopillar-structures functionalized with RDG (arginine – glycine – aspartic acid, a cell binding domain sequence of fibronectin, capable of interacting with $\alpha 5\beta 1$ and $\alpha V\beta 3$ integrin-cell surface receptors), prove to be particularly suitable for *in vitro* improving cell functional features (7).

Micro/nanometer scale-based polymeric structures may be obtained by several techniques including *thermodynamic process* of polymeric solutions (gas foaming $\rightarrow$ phase separation $\rightarrow$ freeze drying), *electrospinning* as production process of polymeric nanofibers under high electric potential difference, *nano-optical-photolithography* to fabricate microstructured polymer hydrogel-based scaffolds, *micromolding* as development of soft lithography (8-10).

In the field of the tissue engineering, anyway made nanobiostructures may be used as *bottom-up-approach* to obtain, through modular combination of cell-laden polymeric microgel subunits, 3D-complex tissue architectures such as bladder multilayer wall, or as *top-down approach* to create micro-/nanoscale structures, such as a microvasculature, within an existing complex scaffold construct (10).

In vivo tissue engineering, nanoscaled features of ECM-based scaffold material surfaces play an important role as favourably affecting cell behavior and neotissue morphogenesis (11).

Even the bioreactors may be improved by nanometer-scale vibration generators to favourably influence, through mechanical nano-stimuli – able to modulate the expression of genes that regulate cytoskeletal structure assembly and dynamics – the cell shape, besides the cell adhesion to scaffold material (12).

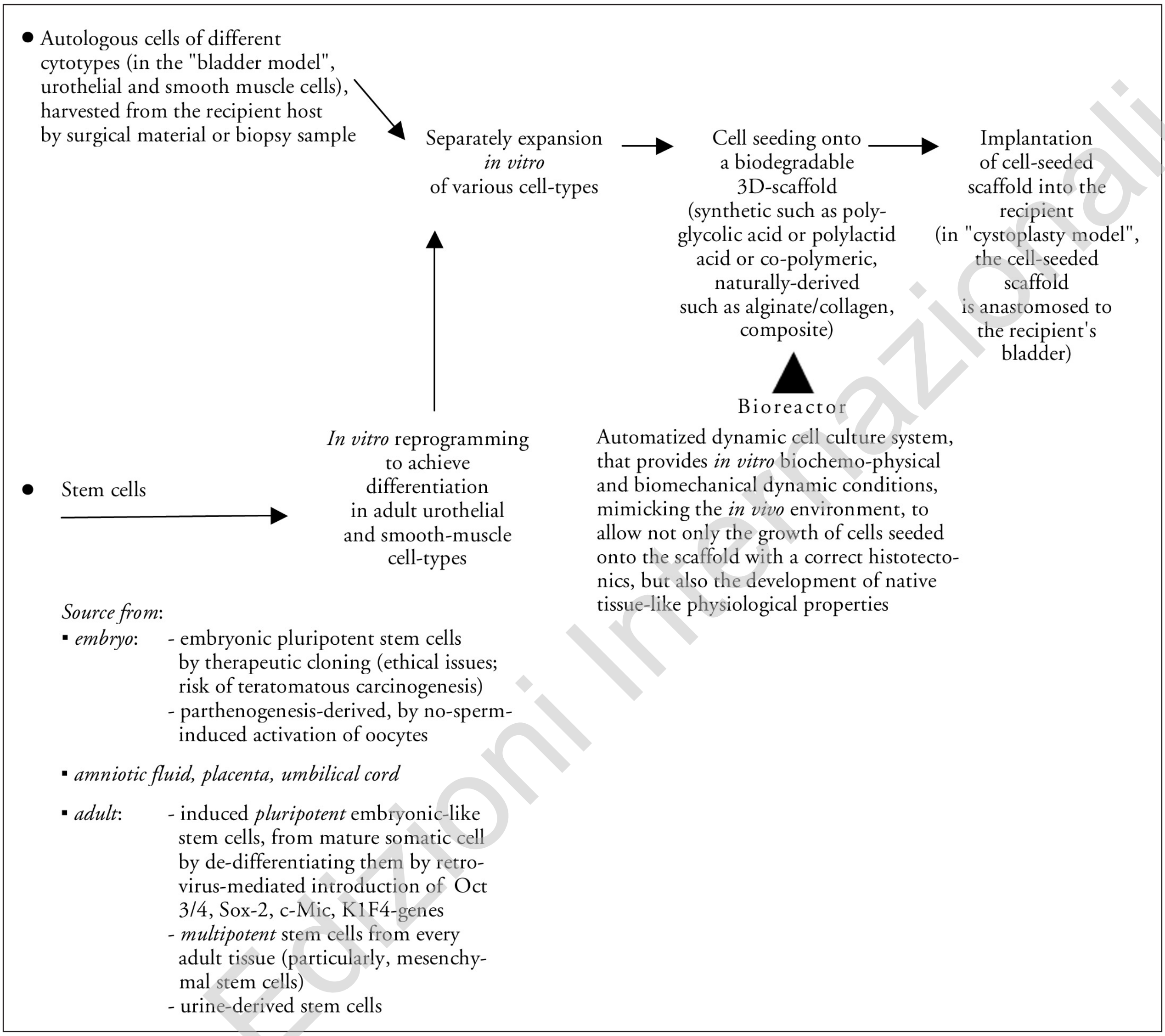
Stem cells and nanotechnology-based tissue engineering

Specific cell-lineage differentiation of stem cells may depend on different, either soft or hard, biomaterial scaffold surface together with its particular features such as nanostructured geometry (nanodots, nanopits, nanorods, nanopillars) and roughness. So, to mimic the native structural features of extra-cellular matrix (ECM), the electrospinning process has been used to produce polymeric nanofiber-based biomaterial (e.g., poly-3-hydroxy butyrate-co-3-hydroxyvalerate, PHBV) fibrous scaffolds able to enhance the growth of bone marrow-derived mesenchymal stem cells, moreover the different nanofiber arrangement/orientation influencing distinct effects on stem cell differentiation by variously driving cytoskeletal structure and dynamics (13, 14).

Since the specific lineage-differentiation of stem cells requires a well modulated spatial-temporal presence of different growth factors and cell signalling molecules, the biomaterials may be provided with nano-/micro- particles able to control the release rates of such molecules (15-17). In this regard, supply of scaffold with bioactive factors prior to implantation can promote, just it has been placed into the host, an appropriate *in situ* host stem cell/progenitor cell recruitment and differentiation (18, 19).

Synthetic enzymatically biodegradable nanohydrogels, composed of PEG, poly-ethylene glycol, and MMP, matrix metallo-proteinase, have been tuned-up for culture of bladder smooth muscle cells (SMC_S) and human mesenchymal stem cells to achieve a significant

TABLE 1 - CONVENTIONAL TECHNOLOGY OF DE NOVO RECONSTITUTION OF A FUNCTIONAL BLADDER BY TISSUE ENGINEERING.



(mod. from Alberti C. G Chir/J Surg 2011; 32: 345-352)

increase in SMC_S, the supply with appropriate signalling molecules allowing a specific stem cell differentiation (20).

Applications of the nanotechnology to bladder tissue engineering

Different approaches of nanotechnology, such as either nanomaterials self-assembling to coat surfaces of existing conventional scaffolds or the resort to electrospinning to obtain *de novo* fibrous scaffolds, may be properly applied to bladder tissue engineering (21). Particularly bladder smooth muscle cell adhesion to nanostructured polymeric surfaces is significantly enhanced in comparison with that to conventional polymeric materials (Table 1) (1).

Different either *bottom-up* or *top-down* tissue engineering approaches by using nanoscale-based polymeric materials allow to achieve a significant improvement in the functional bladder tissue generation, respectively to create a multilayer bladder wall or, on the other hand, to obtain a microvascular structure within an existing scaffold construct (3, 9-11, 22). Moreover, nanostructured polymeric surfaces of bladder engineered tissue show a decreased calcium stone formation (2).

As far as *bladder tumor surgery* is concerned – bladder replacement after radical cystectomy – nanostructured polymeric scaffolds may allow the fabrication of a bladder engineered tissue more properly than tissue engineering conventional techniques, as enhancing, besides the urothelial and smooth muscle cell/scaffold adhesion, the production of ECM proteins (23). Intriguingly, the dispersion of carbon nanofibers within a polyurethane elastomer-based scaffold seems to inhibit the carcinogenic relapse into the prosthetic material, as it has been shown in bladder tumor animal models, where, after cystectomy, the bladder replacement has been performed by resorting to such tissue engineered organ (24).

In the field of neurogenic bladder, the therapeutic management, a part from both traditional-palliative (clean intermittent catheterization, drug therapy) and electronic device-mediated measures (transurethral bladder stimulation, sacral neuromodulation), the nanotechnology applied to stem cell-mediated bladder tissue engineering, could offer novel chances for bladder augmentation surgery, where conventional tissue engineering techniques have already been clinically validated (25-28).

Conclusive remarks

Nanotechnology, by providing scaffold materials with the property of «directly speaking the language of cells» at nanometer level, has opened new ways – via *top-down* or *bottom-up* approaches – for intriguing applications in the field of tissue engineering technologies (3).

Particularly, nanostructured polymeric scaffolds allow the creation of bladder neotissue more properly than conventional ones – considering both cell-adhesion and growth – moreover showing a decreased calcium stone formation (2, 23). Even more interesting, in a bladder tumor animal model, the dispersion of carbon nanofibers in polymeric scaffold-based tissue engineered replacement neobladder, appears to inhibit a carcinogenic relapse into the bladder prosthetic material (24).

Nanotechnologies applied to bladder tissue engineering with resort to stem cells, can allow new chances for *bladder augmentation surgery* (augmentation cystoplasty) in order to lower, in patients with neurogenic bladder, the intravesical pressure together with avoiding or mitigating the development of upper urinary tract changes such as vesicoureteral reflux and hydronephrosis, a critical situation where conventional tissue engineering techniques have already a clinical validation (25-28).

Facing the future, a full success in the realization of bladder tissue engineering will depend on the progress of the science of nanoscale-based materials and the knowledge of stem cell biology (1, 6, 16, 17, 21, 27). In this regard, also about the tissue engineering of other organs (e.g., considering the trachea replacement), a new approach is gaining ground – instead of the use of decellularized human scaffold seeded with autologous cells, sometimes tending to collapse – properly resorting to tailored nanocomposite scaffold seeded with autologous stem cells (29).

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