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Ann. Ital. Chir., 2014 85: 309-316
pii: S0003469X14022842

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L.D. of Surgical Semeiotics

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Among the regenerative medicine technologies, the tissue engineering has emerged, in recent years, as a prominent tool, particularly given the tremendous developments in the field of synthetic polymer-based scaffolds. Scaffold surface coatings with either extracellular matrix (ECM) proteins or integrin-binding bioactive peptide sequences, such as RDG, proved to be extremely useful to enhance cell adhesion and growth. Nevertheless, about it, excellent effects may be reached by electrospinning-obtained nanofiber-structured synthetic polymer scaffold – such as polyurethane or polyethylene-terephthalate electrospun nanofibers – without resorting to surface-coated adhesion proteins. As for bladder tissue engineering, properly cell-seeded synthetic biomaterial-based scaffolds allow today timely chances to obtain constructs provided with specific bladder native tissue-like both histological-immunohistochemical and functional-dynamic features. Recent bright advances in the tissue engineering research, particularly in the area of materials science – together with increasing availability of suitable bioreactors – and stem cell biology, make foreseeable, in the near future, further technological improvements that might widen the clinical applications of bladder tissue engineering up to whole bladder replacement in radical tumor surgery.

KEY WORDS: Coated biomaterials, Neurogenic bladder, Silk fibroin, Nanotechnology, Stem cells

Introduction

The bladder tissue engineering-related clinical applications may particularly regard either congenital or post-traumatic neurogenic bladder, malformations such as bladder exstrophy, scleroinflammatory bladder coarctation and hopefully could be extended to whole bladder replacement in radical tumor surgery, given that this bioengineering technology can avoid both metabolic and malignant complications that may follow the bladder reconstruction with bowell segments [1-5]. Tissue engineering by synthetic scaffolds reseeded with autologous cells, compared with that one by a decellularized donor organ reseeded with the patient's own cells, exhibit many advantages such as to build an organ tailored, as a shape and size, towards the recipient's anatomy and to avoid decellularization agent-induced severe structural/functional tissue engineered alterations that could affect the long-term prosthetic performance, moreover obviously without the resort to a donor [6,7].

Pervenuto in Redazione Marzo 2014. Accettato per la pubblicazione Aprile 2014
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Outlines of bladder physiological features

The urinary bladder, as a muscular-membranous complex hollow organ, is provided with specific relaxation/contractility cababilities, such that to allow urine storage/micturition performances. Given that the inner multilayer impermeable urothelium acts as transducer of intravesical pressure, whose increases induce a stimulation of P2X3 purinergic receptor-related sensory suburothelial nerves, the cooperative urothelium/smooth muscle-based tissue engineered bladder, compared with the intestinal neobladder, represents a functionally more suitable reconstructive measure [8]. The bladder dynamic patterns result from both chemical, neurotransmitter-mediated, and electrical, ion channel function-related, signal interactions between smooth muscle wall component and intramural various afferent/efferent innervation. The biochemical key condition for smooth muscle cell contraction is the rise in sarcoplasmic free calcium, the inositol 1, 4, 5 -triphosphate acting as an important second messenger associated with specific neuromodulator-induced stimulation of smooth muscle cell selective receptors. Moreover the activation of sarcolemmic voltage-gated-L(long lasting)type calcium-channels is involved in the sarcoplasmic free calcium increase, that, in addition, is enhanced by the *Ryanodine receptors-calcium induced calcium release* (RyR-CICR). The smooth muscle cell relaxation, instead, results from extracell free calcium/cytosol free calcium gradient restoration, following the cAMP-induced activation of both the sarcoplasmic reticulum- and the sarcolemma-proper calcium pumps by means of phospholamban [8,9]. Properly cell-seeded synthetic biomaterial-based scaffolds should offer today timely conditions towards tissue engineering modalities so that obtain constructs suitably provided with functional native tissue-like properties, among which particularly the performance of specific signalling pathways regarding the smooth muscle cell phenotype-related contractility.

Development of synthetic biomaterials

Among the developed synthetic materials for their use in cell-seeded three-dimensional scaffold-based tissue engineering (Table I), the properly biocompatible ones – biodegradable-bioresorbable, nonimmunogenic, nonphlogogenic as non inducing foreign-body reactive responses, bactetial/mycotic colonization-resistant, nononcogenic – must also be able to sustain a surrounding microenvironment where the seeded cell properties, such as adhesion, proliferation, differentiation and migration, might be adequately achieved, particularly by interactions

TABLE I - *Biomaterials to build tissue engineering scaffolds*

ECM-derived natural polymers

- protein-based: fibronectin, collagen, laminin, elastin
- carbohydrate-based: hyaluronic acid, chitosan, alginate, agarose, chondroitin sulfate

Limitations of their use for tissue engineering are due to their poor mechanical properties (lack of control for micro/nanostructure, low controllable degradation) together with potential protein based polymer-induced immune reactions.

Synthesis-obtained polymers, sometimes decorated with nanostructured surface

- poly-glycolic acid, PGA
- poly-lactic acid, PLA
- poly(lactic-co-glycolic acid)co-polymer, PLGA
- poly(vinyl alcohol), PVA
- poly-caprolactone, PCL (sometimes blended with PHBV)
- poly(caprolactone-co-lactic acid)co-polymer, PCL/PLA
- poly-ethylene glycol, PEG
- poly-ethylene glycol-polyurethane. PEG/PU
- poly-ethylene-terephthalate, PET
- poly-hydroxybutyrate-co-hydroxyvalerate,co-polymer PHBV
- poly-butylene succinate, PBS
- poly-urethane (elastomer), PU
- poly-urethane-poly(lactid-co-glycolic acid),co-polymer PU/PLGA
- poly-acrylamide, PA or hydroxy-propyl-methyl-acrylamide,HPMA or even poly-methyl-methacrylate, PMMA
- poly-anhydrides and poly-esters(such as poly-hydroxyvalerate-esters)

Natural and synthetic polymer combination-derived materials, particularly including synthetic materials whose surface may be decorated with ECM or provided with bioactive peptide domain sequences (RDG, IKVAV, YIGSR, etc) to enhance cell adhesion/growth. Indeed, different cell-surface proteins – such as specific integrins, glycosaminoglycans, elastin binding protein – play an essential role as receptors for elastin and its derivatives, so that facilitate cell/scaffold material interactivity.

* (Mod. from Alberti C, G Chir, 2012; 33:435-43)

between transmembrane cell receptor integrins and specific soluble growth factors [6,8-10]. As far to avoid tissue inflammatory reactions induced by certain scaffolds, resort has been recently done to biomaterials endowed with dendrimer-polymers incorporating anti-inflammatory agents such as cytokine blocker glucosamine-6 sulfate [11].

Hydrogels, as highly hydrophilic either natural or synthetic polymer-based three-dimensional materials, can form matrices that, because of their properties – such as physico-chemical/biological tunability and versatility in 3D-construction – resemble the native extracellular matrix (ECM), so effectively using them, particularly as elastomeric either naturally-derived elastin-based or synthetic elastin-like polymers, for tissue engineering applications. Moreover shape memory hydrogels, provided with various thermal or pH stimuli responsiveness, represent an intriguing class of "smart" biomaterials with specific shape-memory peculiarities and self-assemblation besides exhibiting a self-healing capability in case of construct network disruption [12]. From for a long time now use of polymers obtained from naturally-derived alpha-hydroxy-acids – such as either polylactic acid, PLA, or polyglycolic acid, PGA, or even co-polymeric poly (lactic-co-glycolic acid), PLGA, provided with effective thermoplastic properties so that allow the creation of scaffolds tailored to recipients – chances have been subsequently reached to build, by the electrospinning procedure, composite scaffolds made-up of synthetic polycaprolactone(PLC) and collagen. Significantly, collagen, as ECM-derived natural polymer, contains cell adhesion peptide sequence domains such as RDG(arginine-glycine-aspartic acid), able to mantain different cell line-associated phenotypic features [13-17]. Polymer *electrospinning process* allows to draw, from a broad range of viscoelastic polymers, solidified ultrathin polymer fibers, as nanospun textures, at micro/nanometer scale (from 100 micro-to 10 nanometer), with high ratio surface/volume. Without going into thorough technical details, an electric field induces polymer solutions – such as polylactic/polyethylene glycol hybrid polymer or polycaprolactone – to pass, under high potential difference, from a syringe, to a grounded material, thus obtaining distinct micro-/nanometer-scale fibers so that build electrospun fibrous scaffolds provided with high porosity, that can improve both cell-seeded attachment and migration together with both the delivery of growth factors and the in-put/out-put respectively for nutrients/waste products [6,13-15,18-22]. Various nanotechnology approaches, including either self-assembling nanomaterials to coat existing conventional surfaces or the resort to *de novo* electrospun nanofiber-based scaffolds, may be suitably applied to bladder tissue engineering, with the advantage, in comparison with conventional polymer constructs, of a lower calcium stone formation [13,14,21]. Other technological modalities, besides the electrospinning process, to obtain nanostructured polymers, include photopatterning, rapid prototiping by previous CAD (computer-aided design) software, stereolithography, up to bioprinting through various modalities such as inkjet bioprinting and wetspinning [12]. The nanoparticle-manipulated scaffold surfaces – where nanoparticles act as mechano-transducers – can mimic the nanoscale topography of native bladder tissue, allowing cell/scaffold interactions at the same size regime of constitutive cell-proteins, particularly of receptor cell-surface proteins, thus "directly speaking the language of cells" [23-25]. 3D-nanodots-, 3D-nanorods- and 3D-nanopillars array nanostructured polymer scaffolds, functionalized with RDG peptide sequences, have been separately tested to identify the entity of cell adhesion, growth and migration, such cell functional features resulting *in vitro* particularly improved by a polymer scaffold nanopillar-geometry [26]. Carbon-nanotubes and graphene, as well as reinforcing the scaffold structure, can improve the cell tracking and sensing of host microenvironment, significantly proving to be identifiable by either magnetic resonance or optical imaging techniques. Moreover, the dispersion of carbon nanotubes within a poly-urethane elastomer-made scaffold inhibits, as it results from bladder tumor animal models, the carcinogenic relapse, after cystectomy, into bioengineered prosthetic bladder tissue [27,28].

The synthetic polymers development has also involved their integration, in addition to collagen, with other ECM native components, such as fibronectin, laminin and elastin so that reach a significant improvement of many cell type binding to the scaffolds, thus actively supporting the growth of different functional tissues. Quite interestingly, the RDG-biopolymer integration-based 3D-scaffolds are so useful in promoting an effective cell adhesion/growth that advantageously may be used as *in vitro* cancer cell culture platforms, in the field of tumor cell biology research, rather than resorting to *in vivo* tumor xenografts [16-18,29].

Out of thoroughly synthetic biomaterials, Bombyx mori *silk fibroin-based scaffolds*, functionalized with ECM-protein coatings, have been tested as templates for urothelial/smooth muscle cell-seeded bladder tissue engineering, they showing to be, about such purpose, extremely effective, given their structural and mechanical properties (plasticity processing, robustness, biodegradability), to support a murine bladder augmentation. Positive correlations, in small animal models, between histologically evaluated degree of silk fibroin-mediated bladder tissue regeneration and by functionally (cystometry) assessed compliance and capacity of so-built cystoplasty augmentation, make foreseeable clinical applications of such experimental research, moreover given that *ex vivo* organ bath tests showed that reconstructed bladder tissues were able to display adequate contractile responses to KCl, carbachol, methylene-ATP besides the electrical stimulations [31,32].

Nanostructured polyurethane-polylactic-co-polyglycolic acid (PU/PLGA)-made scaffolds – further functionalized

with IKVAV (isoleucine-lisine-valine-alanine-valine)-bioactive sequence domain and/or YIGSR(tyr-ile-gly-ser-arg) laminin-derived pentapeptide to improve cell responses – have been successfully tried in minipig model tissue engineering, therefore it suggesting their use to increase human bladder tissue repair after a partial cystectomy due to noninvasive tumor [22]. However, it has been recently shown that microarchitectural scaffold structures, obtained from either polyethylene-terephthalate(PET) or polyurethane (PU) electrospun nanofibers – matching the size-scale of ECM fibers – result more effective than ECM-derived protein surface coatings, in enhancing adhesion/growth/multilineage development of mesenchymal stem cells [30]. As much again, a nanofiber-structured electrospun poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHPV) -made scaffold, quite mimicking the ECM-related fibrous features, can effectively improve the attachment, growth and differentiation of mesenchymal stem cells [33]. Electrospun nanofibrous poly-caprolactone/polylactic acid (PCL/PLA)-based scaffolds proved to be, in animal models, a considerable support for adhesion /growth of both urothelial and smooth muscle human bladder cells, meanwhile without inducing cytotoxic events, what moreover has been also shown for electrospun bioresorbable scaffolds made up of poly caprolactone blended with poly-hydroxybutyrate-co-hydroxyvalerate(PCL/PHPV) [13,34].

Also other biodegradable nanostructured synthetic polymers, including electrospun polyanhydride nano/microfibers and – even though with restricted suitability – some complex poly-ortho-esters, can be a feasible potential as tissue engineering scaffolds [35,36].

Hydrophobic poly (methyl-methacrylate) PMMA/hy-drophilic poly (methacrylic acid) PMAA complex nanostructured 3D-scaffolds are suitable, given their particular tunability, for an appropriate three-dimensional seeded cells distribution. Moreover, novel synthetic acrylate polymers are able to influence adhesion, growth and differentiation of human embryionic cells in unexpected directions [17,23]. Each synthetic polymer-based scaffold fabrication approach significantly allows substantial opportunities of tailor-feasibility for specific tissue engineering applications when resorting to scaffold computer-aided design/manufactoring (CAD/CAM) [37-40].

Different cell type availability/suitability for cell-seeded synthetic polymer-based scaffolds

Autologous terminally differentiated both urothelial and smooth muscle cells obtained from host vesical tissue biopsy, then dissociated from biopsy tissue sample and expanded in culture to be seeded onto the scaffold, so implantable into the same host, should be the preferential type to use [3,10,12,41]. The tissue-speficic growth of both urothelial and smooth muscle cells seeded onto the scaffold is identifiable by expression of immunohistochemical markers, such as respectively cytokeratin 20/ uroplakins (particularly uroplakin IIIb), typical of urothelium, and cytoskeletal contractile proteins (myosin, alpha-actin, calponin) peculiar to smooth muscle cytotype.

Though the use of autologous differentiated cells results quite advantageous as avoiding the engineered neotissue rejection together with immunosuppressive therapy-indu-

TABLE II - *Typology of human stem cells*

Natural stem cells	
Embryonic stem cells:	*Totipotent:* peculiar to zygote and the earliest embryogenesis (up to morula), are able to generate all three germ cell layers growing into the embryo together with extraembryonic structures such as placenta. *Pluripotent*: from the blastocyst stage to terminal embryogenesis stage, able to generate all three germ cell layers growing into the embryo but not extraembryonic structures.
Fetal stem cells:	Fetal proper stem cells, derived from fetus proper tissues, are *multipotent* giving rise to defined organ-pertaining different tissue-cells generation. The developing fetus-derived *amniotic fluid stem cells* are broadly *multipotent.*
Adult stem cells (progenitor cells):	Derived from different adult tissue sources (bone marrow, fat, skin, etc), have the potential of generating some limited cell types. Bone-marrow *multipotent* stem cells consist of two cell populations, the one, properly hematopoietic, able to generate all blood cells, while the other, instead, named stromal-mesenchymal, able to generate, bone-, cartilage-, fat-cells. Most stem cell types, found in defined niches of all adult tissues, are *unipotent* with lowest, only tissue cell-specific, differentiation potential.
Laboratory procedure-generated stem cells	
- by "therapeutic cloning", through somatic cell *nuclear transfer,* to obtain autologous embryonic pluripotent stem cells. - by "genetic reprogramming" of adult somatic cells, to induce the generation of autologous pluripotent stem cells (iPSC).	

ced adverse effects, however such cell sample availability may often result extremely poor, given too extensive host bladder disease [1,3,10]. Hence, the possible resort to different types of *stem cells* (Table II). Because of their peculiar properties – self-renewal and cell lineage specific differentiation under suitable conditions – stem cells can be an intriguing source of cells for different tissue engineering applications, moreover recent advances in the "smart" synthetic polymer technologies pointing to improve their interactions with host surrounding microenvironment. Indeed, the modalities of stem cell differentiation may result from different texture, either soft or hard, of synthetic polymer-based scaffolds as well as from their surface shape, including porosity(pore size), roughness and, particularly, nanostructure [33,42]. *Embryonic stem cells* (EScells), as further expression of their strong pluripotency, can generate, *in vitro,* so-called "embryoid bodies "(three embryonic germ layer-like aggregations of cells), and, "*in vivo",* can unfortunately induce the teratoma-onset, terefore that suggesting a reasonable limitations to use of such cells in tissue engineering as well as in regenerative medicine [10].

By *therapeutic cloning* (somatic cell nuclear transfer-dependent) laboratory procedure-generated, the autologous embryonic stem cells could result useful in tissue engineering applications, but unfortunately some considerable problems – such as poor efficiency of cloning technique, difficult requirement for human oocytes and, in addition, some ethical preclusions – represent a limitation to their use. Stem cells generated by a different laboratory procedure – through *genetic reprogramming,* which implies the de-differentiation of mature-adult somatic cells – so-called "induced pluripotent stem cells "(iPSC), are provided with ES cell-like self-renewal, besides generating, as well as the ES cells, embryoid bodies *in vitro* and teratomas *in vivo*, but, compared to ES cells, they possess a distinct gene expression signature and show a somatic cell/EC cell mixed epigenetic state.

However, intriguing technological advances in the modalities of genetic reprogramming of adult somatic cells, have allow to obtain completely reprogrammed iPSC [10,43]. Developing fetus-derived *amniotic fluid stem cells* are endowed with both ES cell- and adult stem cell-markers, though avoiding, in comparison with ES cells, the *in vivo* formation of teratomas. Among the *adult stem cells*, some are multipotent while most of them – identified in all adult tissue "niches" – showing only one specific cell differentiation unipotency. Multipotent adult stem cells of bone marrow consist of two distinct populations, the one, "hematopoietic", to generate all blood cell types, whereas the other, "stromal-mesenchymal", generating bone-, cartilage-, fat-, and connettive cells. Nanopatterned poly-hydroxybutyrate-co-hydroxy valerate (PHPV) electrospun polymer-based scaffolds can enhance adhesion/growth/differentiation of bone marrow-derived stromal-mesenchymal stem cells given that particular polymer nanofiber orientations promote specific effects on stem cells by properly driving their cytoskeleton structure and dynamics. Microstructured polybutylene-succinate (PBS) scaffold surfaces significantly improve adhesion and alignment of human adipose tissue-derived adult stem cells, so increasing their usefulness in tissue engineering [6,30,33,42-44].

Conclusion and outlook

The clinical validation of the research on *de novo* bladder tissue engineering by use of both urothelial and smooth muscle autologous cells seeded onto a composite polyglycolic acid-collagen based 3D scaffold, has been successfully reached in patients undergone to augmentation cystoplasty because of end-stage neurogenic, myelomeningocele-induced, poorly compliant/high pressure bladder [45]. Recent bright advances in tissue engineering research make foreseeable a positive expansion of clinical applications, in the near future, of the synthetic polymer-based scaffolds allowing to create person-tailored tissue engineered organs [14,20,22,33,34]. Particularly, more and more significant projects are outlining to develop functional bladder tissue-engineered substitutes that can enable storage of urine and restore native like contractile tissue-mediated micturition, in combination with the support of an appropriate vascular/neural network of the bioengineered construct that might be integrated with host's vascularization/innervation upon implantation [12,46]. Otherwise, for patients suffering from neurogenic bladder-dependent *detrusor/urethral rhabdosphincter dissinergy*, it would be excellent, in my opinion, to tissue engineer an implantable neobladder-rhabdosphincter complex – obviously no liable to spinal cord neuropathy effects – provided with intravesical wall microsensors of bladder distension, to send out, beyond a properly adjustable distension value threshold, modulated wireless e-m signals towards a rhabdosphincterial receiver-converter microdevice, able, in turn, to promote, by a suitable e-m field generation, the rhabdosphincter relaxation during the neobladder contraction. Nanotechnologies are quickly opening great chances in tissue engineering by allowing the control of neotissue growth/morphogenesis at the signal transduction micro/nano scale level, the nanostructured synthetic polymer scaffold surfaces suitably mimicking ECM microarchitectural synthetic conformation [6,23-26,30]. It has been experimentally emerged that three-dimensional nanostructure-based biointerfaces act as a powerful platform to guide cell fate in a controllable and appropriate way [47]. Indeed, electrospun nanostructured fibrous-shaped synthetic polymer-based scaffolds can effectively sustain adhesion, growth and specific differentiation of mesenchymal stem cells without the resort to coatings with ECM-derived adhesion proteins [6,13,33,34].

Furthermore, by resorting to recent nanotechnology research advances – such as those enabled by either ato-

mic force- or scanning tunnelling microscopy or even metalorganic chemical vapour deposition – the improvements in both the control and the manipulation of various materials at atomic/molecular level might widen potential applications of the tissue engineering.
Considerable advances in tissue engineering scaffold fabrication are also emerging from the use of "smart" synthetic polymers, as endowed with large conformational reversible changeability in response to extremely small physico-chemical environmental conditions – such as temperature, pH, ionic strength, electrical stimuli besides glucose concentration – from it resulting a substantial opportunity of tailor-feasibility for specific applications [12,17].
To properly develop tissue engineered organs, pivotal tools are suitably designed *bioreactors,* as dynamic cell culture systems, customizable for various applications, inside that synthetic polymer-based scaffolds/seeded cells are conditioned to physico-chemical-dynamic state peculiar to organ/tissue to be replaced. Particularly, bladder mechanical properties (e. g., wall elasticity, compliance towards different filling pressure) simulating proper modular pressure stimulations in cell culture chambres can affect the growth behavior of both urothelial- and smooth muscle cells, it showing their conformational adaptive changes depending on environmental mechanical conditions [48-50].
Provided with customized features, the CAD/CAM moulded scaffolds, by resorting to either bioprinting or 3D-rapid prototyping technologies, may be particularly appropriate for the tissue engineering of either solid-shape organs(e. g., bone, kidney, liver) or heart/blood vessels [38-40,51-53].
Stem cell biology recent knowledge acquisitions, particularly regarding the human embryonic stem cells and iPSC, opened intriguing chances to generate different neotissues, given that stem cell capability to proliferate, self renew and mature cell-lineage differentiate under occurring signaling molecule/growth factor-based microenvironmental conditions. So, autologous pluripotent either embryonic therapeutic cloning-derived (apart from ethic issues)- or iPSC human stem cells may be an effective alternative cell source for bladder tissue regeneration [10,42,43]. Given the tremendous advances – in the course of last years – of various tissue engineering-related technologies, such as particularly in the field of materials science, together with the improvements pertaining the laboratory-generated stem cells, it's possible to foresee, looking to the next few years, further interesting progress up to whole bladder replacement in radical tumor surgery [54].

Riassunto

Nell'ambito delle diverse tecniche di medicina rigenerativa, l'ingegneria tessutale ha raggiunto, negli ultimi anni, una posizione di notevole prestigio, attribuibile, soprattutto, ai considerevoli progressi nello sviluppo di *scaffold* costituiti da polimeri sintetici. Particolare importanza hanno assunto, per la loro prerogativa di favorire adesione e proliferazione cellulare, gli *scaffold* con superficie ricoperta da matrice extracellulare o, in alternativa, dotata di peptidi bioattivi, tra cui RDG (arginina-glicina-ac. aspartico), atti a legarsi a specifici recettori cellulari(integrine). Peraltro, effetti del tutto eccellenti in tal senso, conseguono all'impiego di *scaffold* a struttura fibrosa, ottenuti mediante elettrotessitura, quali, ad esempio, quelli a base di nanofibre di poliuretano/tereftalato, senza, in tal caso, ricorrere alla dotazione di matrice extracellulare o peptidi bioattivi. Quanto alla ingegneria tessutale della vescica, l'impiego di *scaffold* costituiti da polimeri sintetici, alloggianti cellule appropriate(differenziate o staminali, comunque autologhe), dovrebbe consentire, nel prossimo futuro, l'oppotunità di creare un organo ingegnerizzato esattamente riproducente le caratteristiche morfo-istologiche e funzionali-dinamiche proprie del tessuto vescicale nativo. Brillanti risultati conseguenti a recenti sviluppi nella scienza dei materiali e tecnologia dei bioreattori, nonché nella biologia delle cellule staminali, fanno prospettare ulteriori affinamenti nel campo applicativo dell'ingegneria tessutale, tanto da poterne disporre, quanto prima, per la completa sostituzione della vescica in caso di cistectomia radicale per patologia tumorale.

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