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focus on

Tissue engineering: technological advances to improve its applications in reconstructive surgery

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SUMMARY: **Tissue engineering: technological advances to improve its applications in reconstructive surgery.**

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Background. *Tremendous advances in biomaterials science and nanotechnologies, together with thorough research on stem cells, have recently promoted an intriguing development of regenerative medicine/tissue engineering. The nanotechnology represents a wide interdisciplinary field that implies the manipulation of different materials at nanometer level to achieve the creation of constructs that mimic the nanoscale-based architecture of native tissues.*

Aim. *The purpose of this article is to highlight the significant new knowledges regarding this matter.*

Emerging acquisitions. *To widen the range of scaffold materials resort has been carried out to either recombinant DNA technology-generated materials, such as a collagen-like protein, or the incorporation of bioactive molecules, such as RDG (arginine-glycine-aspartic acid), into synthetic products. Both the bottom-up and the top-down fabrication approaches may be properly used to respectively obtain sopramolecular architectures or, instead, micro-/nanostructures to incorporate them within a preexisting complex scaffold construct. Computer-aided design/manufacturing (CAD/CAM) scaffold technique allows to achieve patient-tailored organs. Stem cells, because of their peculiar properties - ability to proliferate, self-renew and specific cell-lineage differentiate under appropriate conditions - represent an attractive source for intriguing tissue engineering/regenerative medicine applications.*

Future research activities. *New developments in the realization of different organs tissue engineering will depend on further progress of both the science of nanoscale-based materials and the knowledge of stem cell biology. Moreover the in vivo tissue engineering appears to be the logical step of the current research.*

RIASSUNTO: **Ingegneria tessutale: progressi tecnologici al fine di migliorarne le applicazioni in chirurgia ricostruttiva.**

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Premessa. *Ai notevoli progressi nell'ambito della scienza dei biomateriali e della nanotecnologia nonché da approfonditi studi sulle cellule staminali, sono conseguiti interessanti sviluppi in medicina rigenerativa/ingegneria tessutale. La nanotecnologia si identifica con un ampio spettro di discipline intese alla manipolazione nanometrica di materiali diversi onde ottenere strutture che imitino l'architettura nanoorganizzata dei tessuti nativi.*

Scopo. *L'articolo è volto a delineare significative acquisizioni conoscitive in tale ambito.*

Dati emergenti. *Al fine di ampliare la gamma dei materiali di supporto (scaffold), si è fatto ricorso a materiali ottenuti con tecnologia del DNA ricombinante, come proteine simili al collagene, oppure alla incorporazione di molecole bioattive, come la RDG (arginina-glicina-acido aspartico), nella compagine di prodotti sintetici. Due approcci costruttivi, denominati "bottom-up" e "top-down", possono trovare appropriato impiego per ottenere rispettivamente architetture sovramolecolari oppure micro/nanostrutture da incorporare in preesistenti costruzioni complesse. Il ricorso alla tecnica di disegno assistito da computer e confezionamento strutturale (CAD/CAM) dello scaffold consente di ottenere organi personalizzati. Le cellule staminali, date le loro prerogative - attitudine a proliferare, rinnovarsi e differenziarsi in specifiche linee cellulari sotto appropriati condizionamenti - costituiscono un attraente riferimento per l'ingegneria tessutale/medicina rigenerativa.*

Prospettive della ricerca. *Nuovi sviluppi nella realizzazione di organi diversi mediante ingegneria tessutale dipenderanno da ulteriori progressi sia nella scienza dei biomateriali nanostrutturati sia nella conoscenza della biologia delle cellule staminali. Inoltre, l'ingegneria tessutale realizzata in vivo si configura come logico avanzamento delle attuali ricerche.*

KEY WORDS: Biomaterials - Nanotechnology - Top-down/bottom-up approaches - CAD/CAM scaffold - Bioreactors - Stem-cells.
Biomateriali - Nanotecnologia - Approcci top-down/bottom-up - CAD/CAM - Bioreattori - Cellule staminali.

Tissue engineering has emerged to remedy the insufficient number of organ donors, by fabricating in the laboratory transplantable tissues/organs to be, consequently, used by the surgeons.

Over the last two decades, tissue engineering research has reached a great development, especially due to swift

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improvement of biomaterials (1). Significant progress has been achieved in nanotechnologies leading to obtain biomimetic both nano- and micro-structured materials for bioengineering applications (2). Moreover tremendous advances in the understanding of stem cell isolation, culture and controlled cell lineage differentiation have enhanced the chances for neotissue generation techniques (3).

Outlines of tissue engineering technologies

Several established methods of tissue engineering lie in cell seeding onto three-dimensional (3D) porous scaffolds to lead tissue morphogenesis that may either materialize *in vitro* up to achieving a proper functional effectiveness or develop *in vivo* after their implantation directly in the host (4, 5). At the beginning of the tissue engineering, the scaffold has been considered as a mere temporary cell-support for either *in vitro* or *in vivo* neotissue creation. Many both synthetic biodegradable – poly-L-glycolic acid (PLGA), poly-L-lactic acid (PLLA), polyethylene glycol (PEG) - and naturally-derived - either collagen and fibrin or carbohydrate polymers such as hyaluronic acid (HA) and alginate – materials have been used (Table 1) (1, 5-7). Scaffold porosity, with adequate pore size, is required to allow cell-seeding-attachment-migration together with transport of nutrients/waste products and growth factors. Among the biomaterials, fibrin offers attractive applications because this polymer contains various ECM (extra-cellular matrix) molecules that are released during its degradation, thus increasing the tissue regenerative process (8). Indeed, an ideal scaffold, a part from its adaptability to mechanical functional properties of the implant siting, must degrade as soon as the cells generate new ECM, allowing, as much as possible, cell-cell interactions to achieve a programmed tissue-specific morphogenesis (1, 3, 9, 10). About it, a biomaterial scaffold should act as the mature ECM – ECM surrogate – carrying out specific signaling functions, underlain to co-relate gene expression (11). Indeed, ECM multifunctional components – such as laminin, fibronectin and vitronectin – are able to stimulate, through cell transmembrane integrin links, the cytoskeletal filaments, so promoting such signalling pathway activation. Moreover the supply of scaffold with bioactive molecules prior to implantation can induce an appropriate cell recruitment and *in situ* stem cell/progenitor cell differentiation (5). In this regard, an integrated systemic/local technique to release bioactive factors able to recruit host stem cells within the implant and, on the other hand, ehnance *in situ* tissue regenerative process, has been developed, by using substance-P for the systemic purpose and stromal-derived factor-1-α for that local (12).

TABLE 1 - SOME BIOMATERIALS FOR TISSUE ENGINEERING SCAFFOLDS.

Natural polymers	- protein-based:
	- fibronectin
	- collagen
	- elastin
	- carbohydrate-based:
	- hyaluronic acid
	- chitosan
	- alginate
Synthetic polymers	- poly-L-lactic acid, PLLA
	- poly-L-glycolic acid, PLGA
	- poly-hydroxybutyrate-hydroxyvalerate, PHBV
	- poly-ε-caprolactone, PCL
	- poly-ethylene glycol, PEG
	- poly-butylene succinate, PBS
	- poly-acrylamide, PA
	- hydroxy-propil-methyl-acrylamide, HPMA

As fas as polysaccharide ECM components are concerned, particularly the hyaluronic acid (HA), a glycosaminoglycan consisting of repeated disaccharide units of glucoronic acid and N-acetylglucosamine, greatly contributes to vertebrate tissue development, especially to complex machinery of renal organogenesis by modulating the ureteric bud branching from the Wolfian duct together with promoting tubule-epithelial differentiation of metanephric mesenchymal cells (mesenchymal-to-epithelial transdifferentiation), so that it is used as 3D-scaffold materials for *in vitro* kidney engineering (13).

More recently, to widen the range of scaffold-materials available for different requirements of the tissue engineering, novel so-called *smart biomaterials* – showing significant conformational changes in response to small microenvironmental physico-chemical variations – have been devised, including 1) gene-engineering-induced mutants of natural proteins, 2) semi-synthesis obtained materials endowed with specific protein domains, 3) engineered peptide-products self-assembling into a nanofiber shaping (1, 14). As for 1), recombinant DNA technology-generated collagen-like protein, spreaded on PLGA-made scaffold, when seeded with condhrocytes, is more effective than wild type II collagen in making up an artificial functional cartilage. Similarly, thiol-modified HA, as enriched with cysteine-bound functional sites of fibronectin, is able to promote the dermal fibroblast recruitment and proliferation to repair cutaneous wounds in animal models (15, 16). As for 2), a thorough research has shown that the incorporation of bioactive molecule signals, such as RDG (arginine-glycine-aspartic acid), into synthetic scaffold materials, improves the adhesion of different cytotypes to their surface (17). In fact, RDG, a cell-binding domain sequence of

fibronectin, is able to interact with α5β1 and αVβ3 integrin-cell surface receptors (9, 17). As for 3), self-assembling peptides into nanofibers and nanoropes, allow to generate, in the filed of the "bottom-up" technique, a variety of sovramolecular 3D-architectures that, in addition, may present bioactive sequences, such as RDG, to recruit cells at a larger amount than that of wild ECM-corresponding peptide epitopes (1, 18).

The self-assembly lies in self-regulating combination of small individual subunits to form a complex structure and includes, on the basis of the size of the units, both the meso- and micro- scale modalities. In the field of tissue engineering, the *bottom-up method* allows, by microscale self-assembling and microfluidics, the modular combination of cell-laden microgel subunits – building functional units – to fabricate, by exploiting their hydrophilic/hydrophobic interactions, 3D-complex tissue architectures, such as multilayer walls, so mimicking the natural morphogenetic course (9, 19-21). On the other hand, the *top-down method* allows to produce micro-/nanoscale structures - such as a microvasculature - within a preexisting complex scaffold construct, by micromodelling, through soft lithographic procedures, appropriate polymers such as PLGA (20, 22).

Because the introduction of scaffold construct into the body may sometimes induce a reactive inflammatory process - recruitment of macrophages, mast cells and dendritic cells - novel biomaterials, provided with dendrimer-polymers hearing antiinflammatory agents, such as TNF-α (tumor necrosis factor-α) / IL-8 (interleukine-8) cytokine blocking glucosamine-6-sulfate, have been recently developed (19).

Essentials on both the fabrication of polymeric nano-structures and the generation of hydrogels

Polymeric structures at micro-/nanometer scale may be obtained by several techniques, among which particularly *thermodynamic processing* of polymeric solutions (from gas foaming to phase separation and, in turn, freeze drying) to construct 3D-scaffold endowed with nanopore interconnections capable of effective diffusion and release of growth factors (10, 23). Furthermore, *solid free-form fabrication modalities* – such 3D-printing – may be used, by CAD (computer-aided design) modulation, to create polymeric structures with highly reproducible architecture (24). *Polymer electrospinning* is an interesting technique to draw, from a broad range of liquid polymers, solidified ultrathin polymer fibers at micro/nanometer scale (from 100 μm to 10 nm), thus resulting a nanospun-texture with high ratio surface/volume. To enter into some technical details, an electric field induces charged either natural or synthetic polymer solutions (e.g., polycaprolactone, polylactide/polyethylene glycol) to pass, as attracted because of high potential difference, from a syringe to a grounded material, thus generating distinct micro-/nanometer-scale fibers for fibrous morphology-based scaffolds (10, 25-28).

Polymer hydrogels are water-swollen materials characteristically mantaining a well-defined 3D-structure and able to self-assembling under certain conditions due to intrinsic protein domains (genetically engineered protein domains integrated in their structure) that are responsive to biological specific stimuli (4, 29). Because of their significant water-soluble material (drugs, metabolites, growth factors, etc) permeability, they can allow an effective delivery of growth factors, gene materials and/or stem cells, sometimes too fast so that may require the resort to «clay gels», endowed with a significant sorptive capacity, to control their release (30). Synthetic, enzymatically degradable hydrogels composed of PEG and MMP (matrix metallo-proteinase) have been tuned-up for culture of bladder smooth muscle cells (SMC) and human mesenchymal stem cells to achieve a significant increase in SMC growth (enhancement of specific cytoskeletal contractile protein expression, such as α-smooth muscle actin, myosin, calponin, etc) and, through an appropriate signal molecule supply, a specific stem cell differentiation (31).

Several techniques – such as photolithography, micromolding, emulsification and microfluidics – have been applied to the hydrogel generation. In the *optical-photolithographic process*, a thin polymer film is exposed to ultraviolet rays through a mask, whose transparent zones delimit photosensitive-UV and photo-reactive polymer regions. This technique is expecially directed to build microstructured, hydrogel polymer-based, scaffolds and to encapsulate cells into microengineered hydrogels (20). Moreover hybrid hydrogels may be fabricated from either covalenty or non-covalenty links of distinct classes of molecules, such as synthetic polymers linked with bio-macromolecules. Nanofabrication technology by optical lithography may reach a resolution that is in inverse proportion to wave-lenght of light, the highest level resulting reached by using extreme UV radiations. A photolithography-related disadvantage lies in deleterious effects of ultraviolet-rays on cell functions (20). With focused electron beam lithography, both elastic and anelastic electron scattering effects on the resist/substrate, disadvantageously give rise to an enlargement of the resist size compared with the size of the incident probe (32).

Micromolding process may be considered as a development of soft lithography, allowing an easier mould fabrication – by using poly-dimethyl siloxane or other polymers – from precreated silicon wafers, so that obtain different material-based (chitosan, PEG, HA, etc) microengineered hydrogels (20, 33).

Emulsification consists in the fabricating, from a multi-phase mixture of various materials (alginate, collagen, agarose), small aqueous droplets – whose size may be modulated varying its mechanical agitation, viscosity or ad-

ding specific surfactans affecting the each phase surface tension – that are gelled to produce spherical microgels and even cell-laden microgels when cells are added to the aqueous phase (20, 33).

Microfluidics technology allows to create microscale hydrogels, whose size and shape are moulded by using designed microfluidic channels, so that tailor them to specific applications of tissue engineering (20, 33, 34).

Furthermore, different free-form tissue fabrication modalities may be complementarily associated with a *3D-inkjet printing-like technique*, to selectively deliver biochemical factors and living cells into complex structure polymer scaffold (35).

Nanotechnology in tissue engineering

The nanotechnology represents a broad interdisciplinary matter – including physics, chemistry, biology, material science and microengineering – that implies the manipulation of different materials at nanometer level, within the size range of small proteins and even of atoms, to achieve the creation of structures and devices that might mimic micro-/nanoscale-based complex architecture of native tissues.

The nanoparticle-modified scaffold surface – nanoparticles acting as mechanotransducers – can improve cell adhesion and growth, cytotype differentiation and cell function, including cytoskeletal assembly and dynamics, hence, by modulation of intra-cell signals, gene-expression and, therefore, protein synthesis (9, 19). Nanoscale surfacing has been widely applied to stem cell, vascular, neural, bone tissue engineering, biomaterial surface nanostructure and geometry – nanodots, nanorods, nanopits, nanopillars – significantly affecting cell/tissue responses (32, 36). Moreover, also nanometer-scale vibrations may affect cell shape, by allowing the expression of genes which control cytoskeletal assembly and dynamics (37).

Nanostructure – 3D-nanodots, -nanorods, -nanopillars – arrays, functionalized with the RDG-peptide, have been tested to distinctly show the extent of cell adhesion, growth and spreading, allowing to define that nanopillars are more suitable for *in vitro* improving cell functional features (38). Moreover, 3D-hybrid nanostructures, consisting of glycolic acid-chitosan and gold particles, besides their use for drug delivering in tumors or in other pathological sites, may be used as scaffold components in tissue engineering (39). PEG-nanopillar arrays, utilized as platform for culture of cardiomyocytes, allow both filopodia elongation and lamellipodia expansion of such cells (40).

Carbon nanotubes, besides providing the scaffolds with structural reinforcement and the cell adesion, may play, in tissue engineering, an important role as allowing the cell tracking together with sensing of host microenvironment, together with the advantage of identifying them by resort to either optical or magnetic resonance imaging techniques (41). Moreover carbon nanotubes, incorporated into the scaffolds, can supply them with intriguing properties such as electron-conductivity, so that they can perform electrical connections along the myocardial structures together with representing, when hybridally combined with PLGA polymers, a suitable surface for cardiomyocite colonization (40-42).

From a comparison between single-walled carbon nanotubes (particularly ultra-short single-walled nanotubes, US-tubes) reinforcing porous synthetic polymer scaffold constructs and the control only polymer-based ones, it emerges that US-tube nanocomposite scaffolds exhibit, in an animal model (implantation in rabbit, both subcutaneously and into bone) respectively either soft or hard favorable responses at micro-CT and histological analyses, thus resulting improved the osteogenesis and connective tissue organization (43).

Self-assembling nanofibrous ECM-derived hydrogels, obtained from swine myocardium ECM and adjusted as a tissue specific injectable scaffold, have been used for heart tissue engineering in animal models, many similarities in terms of nanofibrous structures resulting from the comparison between native ECM hydrogels and that collagen ones (44).

Though at microscale level creation of *cell-laden hydrogels* by the photopatterning technique is plainly practicable, their 3D-assembly to produce a complex 3D-construct yet remains an important challenge (45), which is why cell-laden microscale hydrogels provided with magnetic nanomaterials (iron oxide gold) have been made to allow their magnetic field-driven spatially assembly into 3D-multilayer architectures (46). On this subject, novel tissue-engineered magnetic fibrin hydrogel scaffolds, containing thrombin and growth factors (particularly basal fibroblast growth factor) conjugated iron oxide magnetic nanoparticles, can perspectively allow not only magnetic resonance detection of implanted scaffolds but also their reloading with bioactive agents, such as growth factors bound to magnetic iron oxide nanoparticles, via magnetic forces (47).

In spite of significant advances, over the past two decades, of bioengineering nanotechnology, some investigations about size-dependent effects of nanodot arrays on cell adhesion, growth and apoptosis in several cell lines, have shown anoikis-dependent apoptosis abnormalities in cultured NIH3T3 cells, that yet could be prevented by the nanostructure pretreatment with fibronectin and collagen (48). Moreover, one mustn't disregard the suspected health impacts of some nanostructured materials such as either the possible carcinogenicity of acrylamide and its metabolite glycidamide, or the supposed phlogogenic and even genotoxic effects of fullerenes and carbon nanotubes, though, in this respect, the data of literature are conflicting and heterogeneous.

Computer-aided-design/manufacturing in tissue engineering: a quick overview

Tissue engineered technologies, provided with CAD/CAM (computer-aided design/manufacturing), are very useful to create patient-tailored scaffolds, that replicate customized geometries, particularly achieved by CT-data post-processing, thus obtaining three-dimensional structure models (49), just available today for tissue engineering applications (24, 50-52).

Recently load-adaptive CAD-constructed framework architectures have been devised to properly build mechanically biomimetic 3D-porous scaffolds in tissue engineering (53, 54) and, in addition, to fabricate functionally stage-graded scaffolds – according to various tectogenic phases – with a multilayer internal structure (55).

Besides both bio-blueprint modelling for 3D-cell/organ printing and rapid prototyping methods (50. 56), 3D-CAD/CAM technology in tissue engineering has been developed to build, by laser polymerization, from photosensitive metacrylamide-modified collagen-derived gels, patient-tailored scaffolds with high resolution surface on the order of 1.5 μm (57). Moreover, rapid prototyping modalities offer the opportunity of obtain, in a CAD/CAM fashion, smart scaffolds with an appropriate custom-designed geometry and an adequate pore network, so that allow thick cell-material/cell-cell interactions (58). Photopolymerization-based CAD-CAM technology, applied to bio-degradable polymers – urethane and diacrylate combined with di-thiols and reactive diluents to obtain optimized photoelastomers – allows to precisely achieve tailored vascular constructs for damaged blood vessel replacement (59).

Bioreactors: their use in tissue engineering and regenerative medicine

In vitro technologies to engineer different tissues and organs have greatly evolved particularly to developing the bioreactor design. Bioreactors allow to study the effects of biological and chemical stimuli together with physical conditions and mechanical forces, mimicking the *in vivo* environment, on basic cell activity, so that guide cell growth up to develop an engineered neotissue endowed not only with a correct histotectonics but also with native tissue-like physiological properties (60, 61).

In the field of the experimental bioreactor technology, to provide complex 3D-bioartificial tissue models with adequate nutrient supply, a biological vascularized carrier structure has been developed by obtaining it from a decellularized small bowel segment where the active microvascular architecture, within the ECM, has been preserved (62).

Besides improving the technologies of vascular tissue engineering, bioreactors may represent a suitable tool to both understanding the tissue regenerative process *in vitro* and developing mathematical models to define the cell growth/remodeling dynamics in tissue engineering, together with allowing the adaptation of the culture parameters to the actual conditions of the neotissue development (63). In this regard, to evaluate quantitatively and non-destructively cell/tissue growth during the culture in bioreactors, a miniaturized optically transparent bioreactor, together with an optically accessible scaffold, has been developed so that allow 3D-morphological analyses and other biological studies (64).

3D-dynamic simulated micro-gravity (SMG) culture technique act as stimulatory environment for stem cell growth/differentiation. NASA-approved rotary bioreactor has enhanced the proliferation of human epidermal stem cells showing higher amount of cell Ki67 in comparison with those cultured in static conditions, moreover better reaching a multilayer 3D-epidermis structure (65).

Mechanical stimuli produced by bioreactors improved by nanometer-scale vibration generators can influence cell adhesion to scaffold materials and the cell shape by modulating the expression of genes that regulate the cytoskeletal structure and dynamics (37).

In cell-based tissue regeneration, to prevent low survival of injected cells prepared by *ex-vivo* culture, an implantable electrical bioreactor – composed of biocompatible teflon cylinder containing a flexible polymide electrode and an implantable stimulator – where human mesenchymal stromal cells are simultaneously cultured and electrically stimulated, can be a good tool to improve the performance of stem cell-based regenerative medicine (66).

Stem cells in tissue engineering

Stem cells (Table 2), because of their peculiar properties – ability to self-renewal and specific cell lineage differentiation under adequate conditions – represent an intriguing source for various tissue engineering applications, the smart biomaterial recent developments pointing to optimize their interactions with the host surrounding microenvironment (67-70). Indeed, the stem cell fate within 3D-biomaterial constructs is influenced by several microenvironmental conditions including matrix chemistry and mechanics, pore-diffusion of signaling molecules and growth factors (3). Particularly stem cell differentiation depends on different, either soft or hard, biomaterial surfaces together with their various features (pore size, roughness, nanostructure) (71).

TABLE 2 - STEM CELL DIFFERENT TYPES.

Totipotent	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	Both the naturally embryonic stem cells, that are able to generate all three germ cell layers growing into the embryo but not extraembryonic structures, and the induced pluripotent embryonic-like stem cells ($iPSC_S$) obtained from mature somatic cells.
Multipotent	Later developed during the ontogenesis, are naturally able to generate only differentiated cells peculiar to their tissue of origin. Multipotent stem cells are present in all adult tissues, thus promoting their regeneration. Nevertheless, under certain conditions, they can produce mature cells not naturally present in their tissue of origin (transdifferentiation process due to plasticity properties).

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

Micropatterned scaffold surfaces of PBS (polybutylene succinate) favourably affect attachment and alignment of human adipose-derived adult stem cells, thus resulting advantageous to tissue engineering (72).

To mimic the native structure of ECM, electrospinning technique has been applied to obtain a polyester biomaterial (e.g., poly 3-hydroxybutyrate-co-3-hydroxyvalerate, PHBV) scaffold, to facilitate growth of bone-marrow-derived mesenchymal stem cells, because particular orientations of nanofibers allow distinct effects on stem cell differentiation by properly driving cytoskeletal structure and dynamics (73).

Moreover, technological development in micro/nanotechnology applications to stem cell use in tissue engineering have been further improved, as far as cardiac tissue regeneration is concerned, by more accurate evaluation at optical imaging (74).

Recent discoveries due to bio-nano-research offer intringuing opportunities of using stem cells in tissue engineering, particularly when they are bound to magnetic nanoparticles which might guide and detect their fate (75).

Conclusions and new directions

Tremendous advances in the field of regenerative medicine/tissue engineering have been achieved in the last two decades, hopefully pointing to a bright future.

Given the serious limitations of 2D-scaffolds on cell/tissue growth, morphogenesis and function development, 3D-porous synthetic polymer made scaffolds, much similar to natural tissue/organ architecture, have been created, thus enhancing the amount of cells which may be seeded onto such constructs (1, 6, 8-10, 14, 21, 76).

Either ECM-derived components or synthetic biomaterials mimicking ECM play an important, both structural and functional, role in releasing biomolecular signals that induce cell growth and migration so that guide neo-tissue morphogenesis and actively promote the development of a functional structure (9-11, 18, 23, 29, 31).

Though hydrogels, as insoluble networks of crosslinked polymers with high water level contents, might allow a favorable microenvironment for cell growth, however they do not are provided with a fibrous, ECM-component-like, structure (e.g., collagen, fibrin), hence the resort to electrospinning process is necessary to obtain, from a wide range of polymers, nanofibers as material for fibrous scaffold (25-28).

Nanotechnologies opened new chances in the field of regenerative medicine/tissue engineering by offering the opportunity to control cell/tissue morphogenesis at the signal-transduction nanoscale level, micro- and nano-structured polymers mimicking ECM micro-architecture (2, 9, 10, 20, 32, 36-44).

Bottom-up fabrication methods, based on molecular self-assembly of microscale building units, are employed to create 3D-complex scaffold architectures, while the resort to top-down approaches, such as soft lithography, is necessary to obtain micro/nanoscale structures such as the microvasculature within the neo-tissue (1, 10, 21, 33).

Solid free-form construction technology (3D-printing, 3D-rapid prototyping) more and more are taken into consideration for the CAD/CAM scaffolds endowed with customized features (10, 24, 49-59).

Essential tools to develop tissue engineered organs are specifically designed bioreactors, inside which the scaffold and cells are conditioned to biochemo-physical and mechanical dynamic conditions that simulate those proper of organ/tissue to be replaced (60-66). Bioreactors may be improved by nanometer-scale vibration generators because such mechanical stimuli can favourably affect cell adhesion to scaffold materials and influence the cell shape by modulating the expression of genes regulating the cytoskeleton structure and dynamics (37).

Stem cell biology, with either human embryonic stem cells or induced pluripotent stem cells ($iPSC_S$), has opened new intriguing ways to generate neo-tissues because of their ability to proliferate, self-renew and specifically differentiate under appropriate microenvironmental conditions. To control stem cell/scaffold interactions, analogs of ECM components – such as fibronectin binding domain RDG – may be incorporated into the scaffold so that regulate, together with surface elasticity/roughness, stem cell-adhesion, -morphogenesis, -growth and -differentiation. Since the specific lineage-differentiation

of stem cells requires a spatially and temporally well modulated presence of different factors (signalling molecules, growth factors), biomaterials may be provide with nano-/micro-particles to control the release rates of such molecules (3, 10, 17, 68-75, 77). In this way is directed an approach aimed to *write* molecular signals on scaffold substrate by «2D-pen technology» that employs an appropriate AFM (atomic force microscopy) tool (78). Even a multicellular 3D-constructs generation, with 3D arrangement of living cells – mimicking native cell cohesion and tissue morphology – is today achievable by the use of laser-assisted bio-printing (79).

Genetic engineering, by resorting to recombinant DNA-technology, allows to create various ECM protein-substitutes – such as collagen-like protein or elastin-mimetic protein triblock co-polymers – that, because of their mechanical features and viscoelastic properties, could offer bright opportunities of application in the field of tissue engineering and regenerative medicine (1, 15, 16, 80)

The *in vivo* tissue engineering represents the hopped logical step of current technological advances, by resorting to *smart scaffolds* that, just strewn with autologous stem cells, might be put where, into the body, the regeneration of either damaged or removed organ is required, stem cells themselves recruiting specific body-cells by involvement of different growth factors – fibroblast growth factor-2, vascular endothelial growth factor, transforming growth factor-β1 – and cell biomolecular signals, orchestrating the complex tissue regenerative process (6, 19). Because the *in vivo* tissue engineering approach based on decellularized human cadaveric scaffold intraoperatively seeded with host autologous stem cells or differentiated cells – particularly to replace the diseased trachea – sometimes proves to be unsuccessful given the collapse of the graft likely due to *in vivo* matrix degradation, it follows that the resort to tailored bioartificial nanocomposite scaffold seeded with autologous stem cells might be more properly taken into consideration (81).

Furthermore, important advantages over conventional approaches may be offered by particular implantable cell-free biomaterials (e.g., alginate/HA polysaccharide microengineered hydrogels, amphiphile peptide-derived nanofibrous structures) that are responsive to changes in cell microenvironment and partecipate in active ECM remodelling (3, 9, 19, 20). Nanoscaled features of ECM-based scaffold material surfaces play an important role *in vivo* as influencing cell behaviour, hence neotissue morphogenesis and function (82).

The success in the realization of *in vivo* reconstitution of highly vascularized/cell hierarchically structured tissues will depend on advances in nanoscale materials science and stem cell biology (10, 19, 83).

Intriguingly, an efficient various gene transfection modality on the tissue scaffold by using gelatin-functionalized polycaprolactone film surface – aimed to enhance the cell-adhesion – electrostatically absorbing a cationic vector/plasmid DNA complex, is potentially advantageous to direct cell growth and functions within tissue scaffold construct (84).

A suitable consolidation of all above materials science measures will foreseeably fulfil "the dream of reproducing fully functional tissues" (85), so that allow surgeons to have available tissue engineered transplantable organs (86).

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