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Tissue engineering as innovative chance for organ replacement in radical tumor surgery

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Abstract. – BACKGROUND: Different pathological conditions such as congenital organ absence, severe organ injuries, end-stage organ failure and malignancy-related organ removal, have few effective therapeutic options a part from a whole organ transplant, that, however, often meets with a serious shortage of suitable donor organs.

AIM: The purpose of this paper consists in highlighting what the novel tissue engineering approaches might help to solve such problems.

EMERGING CONCEPTS: A recent approach in tissue/organ engineering, particularly to build bioartificial airways, is the procedure of decellularizing a whole donor organ to obtain a complex 3D-biomatrix-scaffold mantaining the intrinsic vascular network, that is subsequently recellularized with recipient's autologous organ-specific differentiated cells or/and stem cells, to build a potentially functional biological substitute. Such strategy has been clinically used to replace organ in trachea/broncus tumor patients. In another approach, mainly used to construct a bioartificial urinary bladder tissue, different types of either biodegradable synthetic polymers or naturally-derived matrices or even polymer/biomatrix-composite materials are used as scaffold for either cell-free or autologous cell-seeded tissue engineering procedures. So far, such technique has been mainly used to make an augmentation cystoplasty in patients with end-stage poorly compliant neuropathic bladder or in exstrophic bladder subjects.

FUTURE PROSPECTS: Intriguing developments in biomaterial science, nanotechnologies, stem cell biology, and further improvements in bioreactor manufactoring will allow to generate, in the near future, tissue engineered organs that, as for structure/function so the native one-like, might represent the optimum solution to replace organs in tumor surgery.

Key Words:
Trachea, Bladder, Biomatrix, Biomaterials, Nanotechnology, Stem-cells.

Introduction

A quite recent intriguing work points out that a rat subtotal cystectomy can elicit an early strong multilayer bladder wall regeneration – resulting in fully morpho-functional bladder reconstitution – that is unique among mammalian organ systems as it seems to peculiarly mimic salamander limb regeneration via *blastema formation* (cell dedifferentiation, epithelial-mesenchymal transdifferentiation, tissue-specific progenitor cell expansion) rather than the quick mammalians liver *compensatory hyperplasia* following a large loss/surgical remotion of hepatic tissue[1]. The last occurence recalls the mythologic Greek story of Prometheus – god of fire – whose liver, because of punishment inflicted on him by Zeus, every day was torn by an eagle and every night re-grew.

Unfortunately, such rodent bladder wall strong regeneration modality is little natively embodied in the humans where the ensuing result of a large partial cystectomy, without an appropriate patching cystoplasty, is a severely limited capacity-characterized uncompliant bladder. What also occurs for either damaged or surgically excised other hollow organs – among whose the complex tissue structured trachea – that's why different tissue engineering technologies may be helpful to construct native organ-like morpho-functional integrity endowed substitutes particularly to replace organs in surgical oncology.

As for the tissue engineering strategies mainly accomplished to build replacement organs, quite paradigmatic are those respectively concerning either trachea or bladder tissue fabrication.

The design of variously shaped tissue constructs – such as flat (skin), both nonviscus tubular structures (male urethra) and viscus hollow organs (bladder, trachea, vagina) or complex solid organ architectures (kidney, liver) – needs, anyhow, biomaterial-made/ECM (extracellular matrix) scaffold, autologous either mature or stem cells, specific growth/differentiation factors.

Tissue Engineering Main Procedure to Build Airways

The main approach to construct an airway tissue/organ consists in resorting to natural extracellular matrix, obtained by decellularizing a

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donor trachea, because synthetic biodegradable polymer-based scaffolds – such polyglycolic and (PGA)-, polylactic acid (PLA)-, coPGA/PLA – proved inadequate to clinical applications[2]. Indeed, at first, a decellularized human donor wind-pipe, suitably repopulated via an appropriate *ex vivo* bioreactor, with cultured autologous respiratory epithelial cells (REC) and bone marrow-mesenchymal stromal cell-derived chondrocytes, was successfully used as replacement graft of critically diseased main bronchus[4] (Figure 1).

Intriguing improvements of such technique have been promptly carried-out by shortening the decellularization time of the human donor explanted trachea, hence by intraoperatively seeding that with autologous REC and bone marrow-derived monocytes and then resorting to recipient's own body as *in vivo* bioreactor[5]. Such innovative quick procedure – mainly *in vivo* trachea tissue regeneration on implanded recellularized donor biomatrix-scaffold – was subsequently adopted in nine patients with serious-either congenital or acquired, particularly malignant diseases of airways. Among these patients, a partial collapse of the scaffold occurred in three cases, thus it entailing proper refinements in different phases of this approach[6,7]. Particularly, it has been shown that the chemical-enzymatic decellularization modalities of donor trachea can induce a biomatrix critically decline in soluble type II collagen and glycosaminoglycans (GAG) content, thus compromising the mechanical integrity of the tracheal scaffold[8].

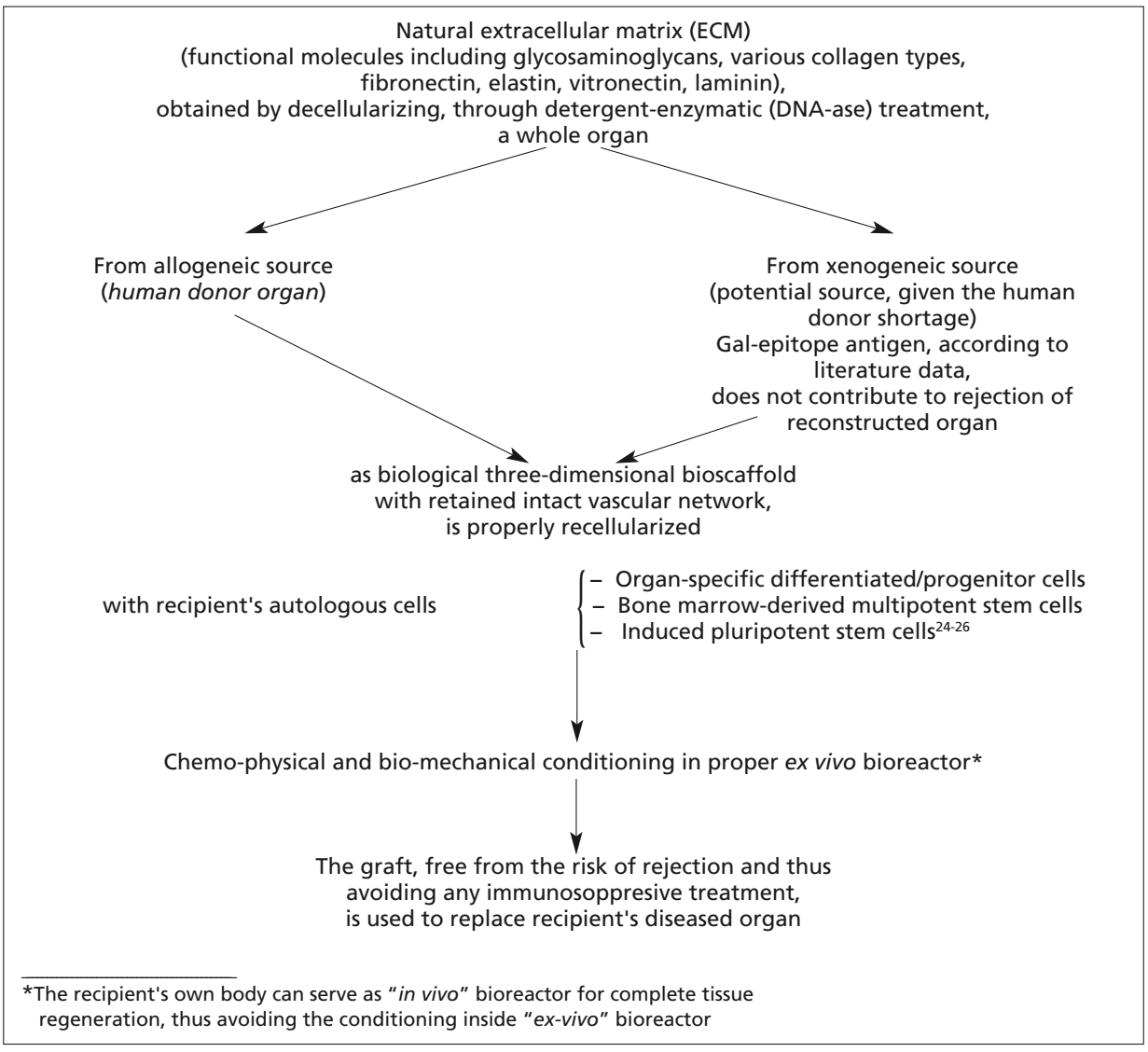

Figure 1. Tissue engineering strategy from naturally-derived extracellular matrix-based scaffold.

Given the above biomatrix-related problems and, in addition, the difficulties in obtaining suitable donor organs, the attention has been turned to fabrication of tailored tracheal/bronchial-shaped nanostructured polymeric scaffolds, endowed with native tissue-like mechanical features, properly *ìn vitro* seeded with autologous REC and bone marrow-derived stromal cells, via *in vitro* bioreactor, to reach a phenotipic and functional appropriate maturation[3,9,10]. Such *nanocomposite scaffold*, seeded with autologous stem cells, has been successfully implanted into a subject suffering from recurrent primary tracheobronchial tumor, together with enhancing the *in vivo* airway wall regeneration by use of specific bioactive agents and growth factors, directed to attract, within the scaffold, peripheral and local progenitor/stem cells[10].

Even though considering these innovative technological contributions, the already proven procedure of using a decellularized donor tracheal scaffold, seeded with autologous bone-marrow mesenchymal stem cells plus autologous epithelium patches, and properly boosting the angiogenesis and chondrogenesis within the engineered tissue, has been clinically validated in recent 2 year follow-up study[11]. Thus it is strengthened that to reach clinically successful whole airway replacement by tissue engineering technologies, a 3D-bioscaffold, composed of nonautologous source-derived biomatrix, hence recellularized with autologous either stem cells of differentiated cells, together with using specific bioagents to boost a proper stem cell recruitment/mobilization, represents an effective tissue engineered prosthesis[3,12].

Intriguingly, an experimental investigation has been carried out, in a pig model, to study *in vivo* regeneration of decellularized pig trachea – without recellularization before its transplantation – by intraoperatively treating that with specific growth/differentiation factors and mononuclear cells, and then by using the recipient pig's own body as *in vivo* bioreactor. The post-operative controls showed quick trachea *in vivo* reconstruction (just after two weeks), provided with respiratory tissue, thus validating such *in vivo* airway tissue engineering strategy[13].

Tissue Engineering Main Procedure to Build the Urinary Bladder

To avoid both metabolic and malignant problematic complications of the intestinal neobladder – however it still remaining the gold standard of the urinary diversion following radical cystectomy in bladder tumor patients – different solutions have been proposed to get an artificial neobladder: from alloplastic nonbiomaterial-made bladder – by using polyurethane, polytetrafluoroethylene, silicon rubber – that are discarted because of nonbiodegradable material-related negative outcomes, to, more recently, tissue-engineered bladder by using autologous urothelial- and smooth muscle-cells seeded onto biocompatible either synthetic (PGA, PLA, coPGA/PLA) or natural ECM scaffolds, the just-mentioned synthetic ones showing termic stability under various body temperature conditions together with both enzymatic-hydrolitic biodegradability and absence of toxicity (Figure 2)[14-23].

Current technologies to obtain an augmentation cystoplasty with a bladder tissue engineered include both *unseeded* (cell free matrix) and *seeded* (cell matrix) modalities. The *unseeded method* consists in anastomosing a naturally-derived acellular matrix – particularly collagen sponge, small intestinal submucosa (SIS), bladder acellular matrix (BAM) – with host bladder to induce, *in vivo*, a natural biomatrix-guided vesical wall cell-repopulation from both urothelial and smooth muscle cells, arising from the neithbouring native bladder tissue or/and even from the ureters when directly implanted into biomatrix[16-19]. Porcine urinary bladder matrix (UBM) seems to be provided with significantly higher potential, compared with SIS, to support the growth of human urothelial cells[20]. The *seeded method* is characterized by seeding cultured autologous urothelial and smooth muscle cells, obtained from the host urinary tract tissue, onto either synthetic biodegradable material (PGA, PLA, co-PGA/PLA)- or natural matrix (collagen, SIS, BAM)-made scaffold to *in vitro* build a morpho-functionally suitable replacement tissue[21-23] (Figure 2). Anyway made, tissue engineered bladder must to have peculiar native bladder-like properties, some of them properly urothelium-related – urine permeability barrier, intravesical pressure-sensitive transducer function, effective ECM-cytoskeleton-nuclear matrix interactions by both various shuttle molecule/growth factor-mediated chemical cell signaling and extra/intracell microelectric current/bioresonance biophysical connections – while others smooth muscle layer-linked such its specific dinamics[14,27-30].

Getting down to tissue engineering clinical implementations, bladder augmentation with *acellular matrix*, in exstrophic bladder patients,

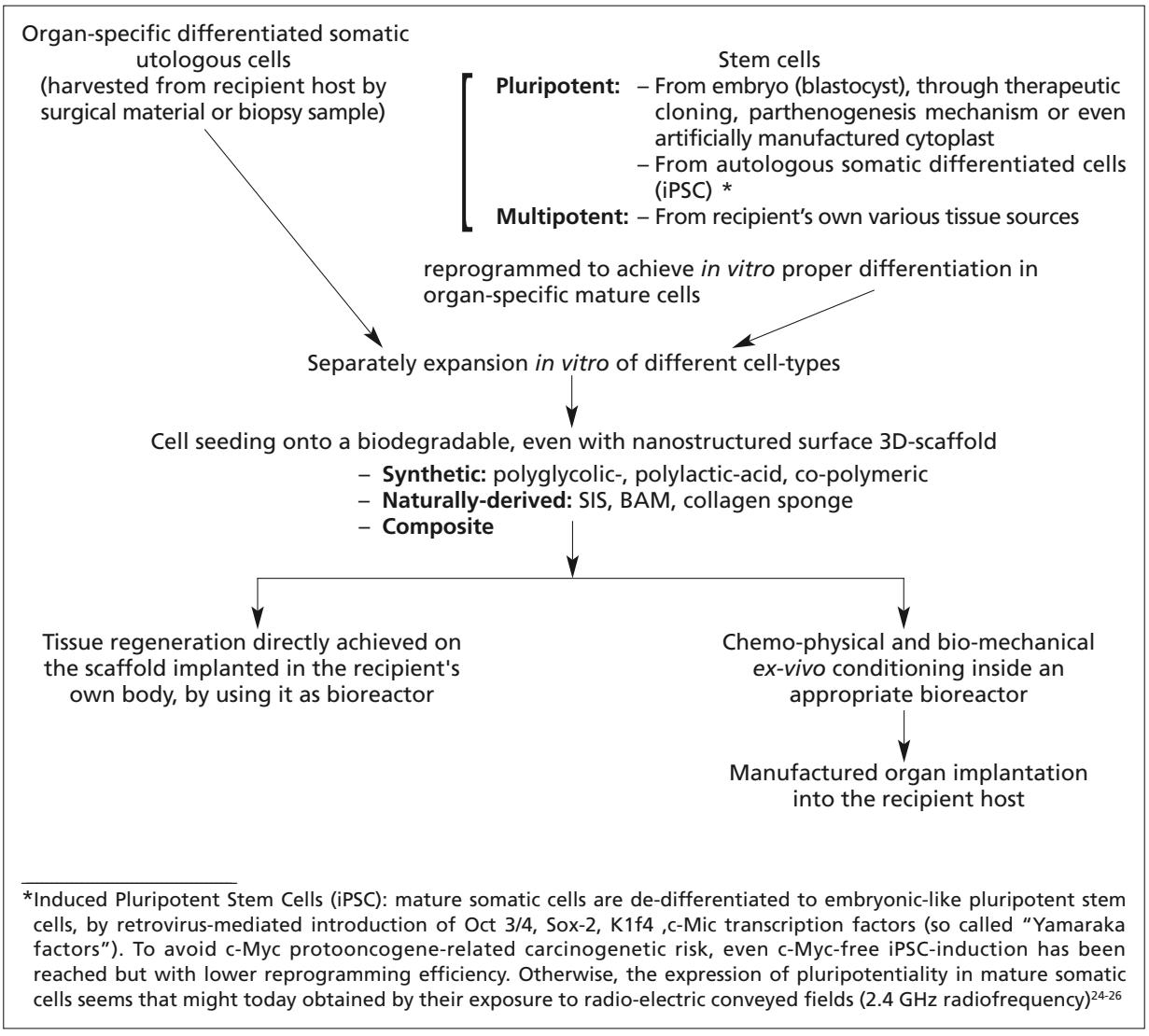

Figure 2. Tissue engineering strategy from biodegradable polymer-based scaffold.

failed to provide long-term effective outcomes as for both bladder capacity and compliance together with urinary continence[31]. Instead, a *cell seeded composite 3D-bladder engineered tissue*, made up of collagen-biomatrix plus polyglycolic acid, has been implanted, with omental drap, in patients with end-stage neuropathic poorly compliant bladder to successfully reach an augmentation cystoplasty[32,33].

Because of potential limitations of *in vitro* urinary tract-derived autologous cells – possible complications due to invasive tissue biopsy, precariousness of specimens from a widely unhealthy organs, low *in vitro* proliferation of adult organ-derived cells – the resort has been taken into consideration, for cell-based bladder engineering, to either pluripotent/multipotent *stem cells*, endowed with self-renewal and differentiation in various tissue-specific cell-lineages, or proper progenitor cells. Among pluripotent stem cells, the interest in the induced pluripotent stem cells (iPSC) is more and more increasing[24-26,34-37] (Figure 2). On this subject, even amniotic fluid-, placenta-, umbilical cord-derived stem cells and, in addition, those isolated in urine samples collected from upper urinary tract, could be an alternative cell source to build a tissue engineered neobladder for vesical tumor patients undergone total cystectomy[38,39].

Recent advances in the field of nanostructured biomaterials – *polymer surface nanofacturing* – that can mimic the nanoscale topography of native tissues with optimum cell/scaffold interactions, together with the innovative design of vari-

ous *smart biomaterials* (materials provided with specific protein domains such as RDG, arginine-glycine-aspartate, a cell-binding domain of fibronectin; gene-engineering-induced mutants of natural proteins; etc), and the resort to *hydrogels for bioprinting* applications, have significantly enhanced the tailored tissue engineering-related challenges[38,40-51]. As for as *gene-engineering* – gene (nucleic acids) delivery to a variety of cell populations such as nature cells, progenitor cells, stem cells – to specifically direct neotissue formation, physical methods, such as transfection by electroporation, rather than viral vectors, can induce highly effective results[52].

With reference to above nanotechnology innovations, it has been proven that bladder smooth muscle cell adhesion to *nanostructured polymeric surfaces* is significantly improved compared with that to conventional polymeric materials[53,54]. Moreover, in animal models, nanostructured polymeric surfaces of bladder wall engineered tissue prove to be refractory to calcium incrustation and calcium stone formations[55]. What's more, in a bladder cancer animal research study, specifically regarding the post-total cystectomy replacement bladder tissue engineering, the dispersion of carbon nanofibers within a polyurethane elastomer-based scaffold can inhibit the tumoral relapse into the bladder prosthetic construct[56].

Just recently, great promises of tissue engineering strategies, for bladder tumor-related surgical organ substitution, have been highlighted, although underlining a number of still unsolved problems, such as particularly the difficulty in obtaining, from different sources, a population of effective smooth muscle cells that may be functionally assimilated to those of native bladder[57,58].

Further advances to build, by tissue engineering, a neobladder including the trigone/vesical neck, such that might be implanted, after radical cystectomy, in bladder cancer patients, could be likely achieved by resorting to a decellularized donor whole bladder biomatrix – retaining distal ureters and vesical neck together with preserving wall vascular network – then repopulating it with various source-derived autologous cells, to reach a fully functional organ substitute[30,38,40,62], particularly with regard to the attainment of a properly contracting so-made smooth muscle layer.

Current Research Focus and Future Prospects

For patients suffering from seriously injured or end-stage diseased organs, it is often necessary to resort to whole organ transplant. Because of critical shortage of suitable donor organs, the tissue engineering technologies represent intriguint strategies to build biological replacement prostheses, that morpho-functionally can mimic native organs[3-6,10-13,21-23,28-30,38].

In the field of organ transplant surgery, clinically validated current tissue engineering approaches essentially consist in two different procedures, one by resorting to a *scaffold composed of naturally occurring extracellular matrix*, obtained by decellularizing an allogeneic (or, in prospect, xenogeneic) whole organ, otherwise retaining intact intrinsic vascular network and appropriately repopulated with autologous stem cells/differentiated cells, the other, instead, by using a synthetic polymeric biomaterial (PGA, PLA, co-PGA/PLA)- or, sometimes, a biomatrix/synthetic polymer composite-based scaffold, seeded with autologous organ-specific differentiated cells/stem cells. The former has been mainly used to achieve replacement tissue engineered airways for patients suffering from tracheal/bronchial different diseases, among whose particularly the malignant ones[3-6,10-13,59], while the latter, so far, to accomplish an augmentation cystoplasty in subjects with end-stage neuropathic poorly compliant bladder, thus obtaining an adequate bladder low-pressure capacity to avoid renal damages[21-23,28-30,38], nevertheless without any clinical application of wholly tissue engineered neobladder in bladder tumor radical surgery, hence no relevant literature report, unlike what pertaining to airway replacement in trachea tumor patients. The autologous neourinary conduits, that have been also taken into our consideration more than ten years ago[66] and whose clinical trials are today in course (ClinicalTrials.gov.identifier:NCTO1087697), though potentially directed to eliminate the intestinal neobladder-related complications[15,67] meanwhile simplifying the surgical procedure compared with Bricker's operation, are far from optimizing – as urinary diversion modalities needing an external urinary reservoir impairing the quality of life – the aims of tissue engineering, that should consist of building an orthotopic bladder replacement anastomosed to the urethra or, at least, a continent cutaneous urinary diversion (pouch)[62].

All the more so, tissue decellularization technology by detergent-enzymatic treatment, as providing a natural ECM-based scaffold retaining own vascular network and native structural cues, besides its use for hollow organ engineering, could be also applied to engineered transplanta-

tion-directed whole solid organs (liver, kidney, lung) for the radical tumor surgery (and just in case of end-stage organ failure[60]. Apart from some above-signified issues[10], the decellularization technology, applied to trachea tissue engineering, allows the complete removal of native organ cellularity/antigenity meanwhile, according to recent studies[61], preserving its histoarchitecture, though with significant loss of glycosaminoglycan, and an adequate mechanical strength with good cell-repopulating compatibility from the decellularized matrix[11,61].

As future prospect in the field of surgical oncology, the goal of bladder tissue engineering will be reached by the construction of the whole artificial neobladder, provided with trigone/vesical neck and distal ureters, such that might serve as wholly replacement bio-prosthesis for bladder tumor patients undergone a radical cystectomy[62-65]. So-made bioengineered neobladder could efficaciously avoid – as it as been above-mentioned about the tissue engineering main procedure to build the bladder – both metabolic and malignant complications of the intestinal neobladder[14,15,66,67]. Six years ago, indeed, a careful analysis on perspectively feasible modality to replace bladder, after its malignancy-related total removal, just identified it with a tissue engineering-made neobladder[68].

Facing the future, further discoveries in the field of nanotechnologies, particularly as far as nanostructured polymer biomaterial scaffold surfaces so that better mimic cell/scaffold interactions at the nanoscale topography of the native tissues – thus offering the advantage of «directly speaking the language of cells»[69] – will allow tissue engineering technological developments for organ replacement applications in radical tumor surgery[38,40,44,46,47,50,51,53,54,56,60,70-72].

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