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From nano to macro: enabling nanotechnologies for human organ biofabrication (Electrospun Nanofibers and Hybrid Technique)

Rodrigo Alvarenga Rezende[1,4*], Marcos Antonio Sabino[1,2], Janaína de Andréa Dernowsek[1], Júlia Adami Nogueira[1], Fábio de Albuquerque Vilalba[1], Vladimir Mironov[1], Jorge Vicente Lopes da Silva[1]

[1] 3D Technologies Research Group (NT3D), Center for Information Technology Renato Archer (CTI), Rodovia Dom Pedro (SP-65) km 143,6, Amarais, 13069-901, Campinas, São Paulo, Brazil.
[2] Grupo B5IDA, Departamento de Química, Universidad Simón Bolívar, Caracas, Venezuela. [3]3D Bioprinting Solutions, Moscow, Russia.[4]CERTBIO, Federal University of Campina Grande (UFCG), Campina Grande, PB, Brazil.
**Corresponding author: rrezende@cti.gov.br*

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ABSTRACT

This review proposes to present how materials at nanolevel scale can contribute to the development of three-dimensional (3D) structures, human tissues, and organs which have macrolevel organization. Specific nanomaterials such as nanofibers and nanoparticles are presented and discussed in their application for biofabricating 3D human tissues and organs. The concept of self-assembling magnetic tissue spheroids as an intermediate mesolevel structure between nano and macrolevel organization and building blocks for biofabrication in dual scale level of complex 3D human tissues and organs is detached. The challenges and perspectives of employing nanomaterials and nanotechnological strategies in the biofabrication were also traced.

1 Introduction

It is essential to understand how recent advances in nanotechnology on nanolevel can enable and enhance progress in organ biofabrication on a macrolevel.

Biofabrication could be defined as an application principle of engineering and information sciences for automated robotic bioassembly of living 3D human tissue and organs (Mironov *et al*., 2009a; Guillemot *et al.*, 2010; Hinton *et al.*, 2017). In other terms, biofabrication is a biomedical application of additive manufacturing technology or computer-aided additive fabrication (Bracaglia *et al.*, 2017). The concept of biofabrication was reappraised and redefined by Groll *et al.* (2016) as the automated generation of biologically functional products with the structural organization of living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bioassembly and subsequent tissue maturation processes.

The emerging biofabrication research field is focused on using robotic automated engineering approaches in tissue bioassembly. Additive Manufacturing has transformed the global industry since automation and robotic techniques started to be employed with very high accuracy and repeatability. Further the hardware, software incorporates algorithms for optimizing the process assuring high quality products.

The recent state of tissue engineering and the biofabrication could be compared to the situation of the microelectronic industry before and after the introduction of automated robotic technologies for fabricating microchips and microprocessors. The robotization and automation helped to convert emerging promising technologies into economically feasible industries. Moreover, it is logical to predict that industrial scale engineering of complex human organs is practically impossible without advances in robotization and automation of biofabrication process.

Unquestionably, nanotechnology is one of the most relevant and rapidly emerging technologies of the XXI century (Roco, 2003). During the last decade, the exponential growth of nanotechnology applications in the area of tissue engineering had been strongly observed (Gyles *et al*, 2017; Rabionet *et al.*, 2017). Concerning published contents devoted to tissue engineering field in the last ten years, the number of publications has been also dramatically augmented (Mironov *et al*., 2008a,b; Mironov *et al*., 2009a,b; Lionetti *et al*., 2011; Rezende *et al.*, 2012; Perán *et al*., 2012). A quick systemic analysis consult on

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PUBMED online database regarding the terms “nanotechnology” and “tissue engineering” showed that the number of publications of these fields is crescent over the years (Fig. 1).

The most considerable and non-trivial related to the interface of tissue engineering and nanotechnology is: “how can the use of materials (on nanolevel) enable biofabrication of human organs (on macrolevel)? ”.

One of the most important points in the hybrid process is the wide range of usable materials. The materials can go from metals, ceramics, polymers (natural and/or synthetics) until composites mixing these groups. Looking at the biofabrication aspects ceramics and mainly polymers are highly used. On hybrid processes involving a 3D bioprinter and electrospinning as main topics in this paper, in general, the most common materials applied are alginate, collagen, chitosan, starchs as being the natural ones plus PCL (poly caprolactone), PLA (poly lactid acid), PLLA (poly L-lactic acid), PDLA (poly D-lactic acid), PLGA (poly lactide-co-glycolide) as the synthetic ones.

Therefore, the objective of this review is a presentation of how the most recent advances in the application of nanomaterials in tissue engineering can enable the robotic and automated biofabrication of 3D human tissues and organs which have macrolevel organization. The challenges and prospects of nanomaterials application in tissue engineering and nanotechnological strategies in organ biofabrication will also be discussed.

We would like especially to emphasize that this is not a comprehensive review about nanotechnology in tissue engineering, but rather focused attempt to explore the emerging potential of applications of nanotechnology to predominantly organlevel of tissue engineering or, more specifically, for organ biofabrication and how this nanoscale contribute to createthese three-dimensional structures that have a macroscale.

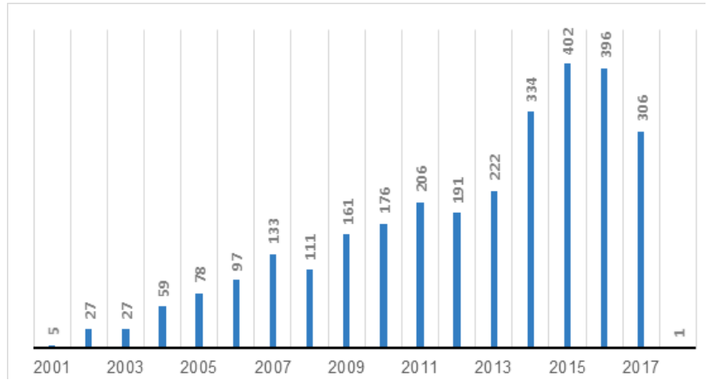

Figure 1 - A chart illustrating the times the terms “nanotechnology and tissue engineering“ appear on PUBMED database

2. Nanofibers

The electrospinning technique has appeared as a relatively simple and scalable nanotechnological method for the generation of nanostructured scaffolds (Hidalgo *et al.*, 2013).

Electrospinning allows controlling the diameter of the spun fibers and can produce nanofibers and scaffolds that mimic the nanostructure of natural extracellular matrices (ECMs), as confirmed by a more efficient vascular cell attachment and spreading (Lee *et al*., 2007).

The general aspects of the electrospinning technology have been substantially reviewed elsewhere (Murugan and Ramakrishna, 2006; Barnes *et al.*, 2007; Murugan *et al*., 2007a; Mironov *et al*., 2008a,b,c, Hidalgo *et al.*, 2013; Braghirolli *et al.*, 2014). Considerable attention will be given on the usage of the electrospinning technology in the field of vascular tissue engineering (Kitsara *et al.*, 2017). A wide range of synthetic polymers has been successfully applied in the electrospinning technique. Furthermore, electrospinning of natural proteins, such as collagen and elastin, either alone or as in blends with synthetic polymers, has been published (Boland *et al*., 2004; Lee *et al*., 2007; Aguirre-Chagala *et al.*, 2017). However, the reported stability of vascular scaffolds created by electrospinning of merely natural proteins had not reached desirable levels yet (Ma *et al.*, 2008).

Although dense nanofiber meshworks provide excellent conditions for cell attachment and the spreading of endothelial cells on the luminal surface of the scaffold (Bondar *et al*., 2008), they also preclude effective cell migration into the scaffold and thus impede smooth muscle cell migration and the sequential formation of muscular layers inside the vascular tissue engineered constructs. Electrospinning allows the fabrication of a wide variety of nanofibers and nanostructured scaffolds with special characteristics and functionalities. The obtained nanofibers vary in shape, size and composition: they can be solid, composite, hollow, porous, decorated, helical and branched. This diversity of possible electrospun nanofibers offers interesting opportunities for the enhancement of vascular-scaffold functionality. For example, the hollow nanofibers and nanoshells created by a coaxial extruder, as well as composite-coated or decorated

auricular implant. Another promising direction is using drug-eluting nanofibers which will enable fabrication cost-effective cell-free hybrid dual scale scaffolds with optimal mechanical properties, superior biocompatibilities, and capacities for induced endogenous regeneration. The cell-free complaint electrospun scaffold has been shown to be promising in case of vascular tissue engineering (Wu *et al.*, 2012). Drug-eluting electrospun vascular scaffold has been also reported recently (Innocente *et al.*, 2009). It has been shown recently that cell-free bioprinted drug-eluting scaffold can induce the formation of cartilage *in vivo* (Lee *et al.*, 2010). Thus, cell-free drug-eluting dual scale biomechanically optimal and biocompatible scaffold fabricated with using hybrid FDM and electrospinning technology with capacities for induced endogenous regeneration could lead to the development of economically cost-effective implants and scaffolds. In case of using cell-free scaffolds or even non-biodegradable complaint and biocompatible implants fabricated by above described hybrid dual scale scaffold technology, it will be much easier to get required regulatory approval, and an accelerate desirable clinical and commercial translation.

Moreover, the other significant advantage that is achieved with this type of hybrid system is that different types of materials can be mixed, and 3D structures can be created where layers of materials can be deposited using the two techniques, that is, it can be used the material for FDM deposition and another for electrospinning. Also, a layer can be made by FDM and then place a layer using electrospinning and create sandwich-like structures, which may even allow obtaining systems with different rates of degradation (Fig. 4).

Other advantages that have been experienced through electrospinning are the incorporation of nanoparticles of inorganic compounds such as hydroxyapatite (HA), or metal particles such as silver (Ag). In the polymer solutions, these nanoparticles are dispersed, for which use can be made of some dispersing agent that does not allow their agglomeration, and they are dispersed through the fibers. In addition, given its nature, it is possible to increase the conductivity of the solution and achieve a good integration of the particles inside the fibers, as shown in Figure 5. In this figure, it can observe the external morphology of the fibers by SEM and the inner of these fibers by TEM showing the presence of the nanoparticles (HA and Ag) distributed around the fibers. This is evidence that through this technique, there are many hybrid type systems that can be obtained, in addition to the possible incorporation of drugs or biomolecules in the inner of the fibers as evidenced by several scientific papers reported (Balogh *et al*, 2015).

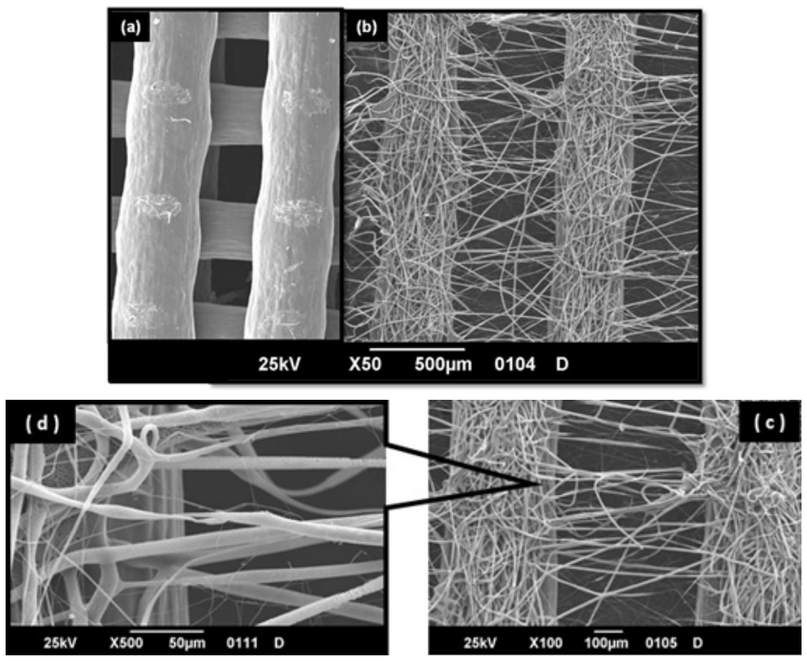

Figure 4 - SEM micrographs of the double scale scaffold. (a) PCL 3D scaffold obtained by FDM, where macropores are shown. (b) Image of the combined system after deposition of PLA (poly lactic acid) micro and nanofibers using electrospinning. These filaments show a preferred orientation and cover macropores created by Additive Manufacturing (FDM technique). (c) and (d) details show the good adhesion of the filaments deposited by electrospinning on the previously created scaffold by FDM. These structures allow not only the combination of scaffolding techniques, but also the combination of polymers. (Images supplied by B5IDA research group, Universidad Simon Bolivar, Venezuela).

nanofibers, could provide additional functionalities, including the capacity to release oxygen and to present growth factors and RGD peptides (Zhu *et al.*, 2017).

However, the full potential of electrospinning for the engineering of the full array of nanofibers with different functionalities remains to be explored. Significant progress has already been made related to the control of the fiber orientation (Murugan and Ramakrishna, 2007b), which is an important step toward the rational design of biomimetic vascular scaffolds. Nevertheless, in our opinion, controlling only the orientation of the nanofiber will probably not be enough. The recapitulation of the entire matrix architecture and the non-linear biomechanical behavior of the natural vascular wall are dramatically crucial. The most exciting advance in electrospinning is the successful one-step rapid fabrication of a vascular scaffold with integrated living cells (Stankus *et al.*, 2006). In these studies, the previously reported methods for the encapsulation of living cells were combined with the electrospinning of nanofibers into one procedure. Further optimization of this electrospinning strategy might offer the greatest potential for rapid biofabrication of vascular-tissue constructs and might eventually eliminate the need for time-consuming and expensive bioreactor-based cell seeding and scaffold cellularization. Although this impressive progress in vascular tissue engineering with the help of innovative electrospinning technologies has occurred, rapid cell integration into scaffolds and their optimal mechanical properties remain the main defiance.

The nanostructured electrospun matrices are an excellent substrate for fabrication of transplantable cell monolayer due to the biomimicking character of nanofibers; moreover, the resulted optimal conditions for cell attachment and spreading could compete with scaffold-free cell sheet technology (Hidalgo *et al.*, 2013). The potential functionalization of electrospun matrices and their transformation into drug-eluting scaffold provides another advantage (Zhan *et al.*, 2017).

On the other hand, dense electrospun nanostructured matrices usually do not present optimal properties for the cell invasion and effective cell seeding. Theoretically, it is possible to fabricate matrices with larger pores using additional spinning of sacrificial fibers or cryo-electrospinning (Kamoun *et al.*, 2017). However, the attempts to increase pore sizes and make adequate electrospun matrices for cell invasion and cell seeding could negatively impact on the material properties of matrices. The efficient solution of these two interdependent problems represents the main challenge in development and clinical translation and commercialization of electrospun technology in tissue engineering. The hybrid composite approach opens possible solution in the fabrication of electrospun matrices with desirable natural-like material properties. The development of electrospinning apparatus with two nozzles (for example, one for fabrication collagen mimicking nano and microfibers and one for fabrication elastin mimicking nano and microfibers) in combination with modified fiber collector with changeable diameter will allow fabricating wavy collagen mimicking nanofibers and biomechanically compliant composite vascular graft.

Coaxial and rotational electrospinning allows the biofabrication of a new vascular graft (Fig. 2). This type of equipment not only allows mixing different types of polymers, or to place layers of different types of polymers, but also allows obtaining different rates of degradation

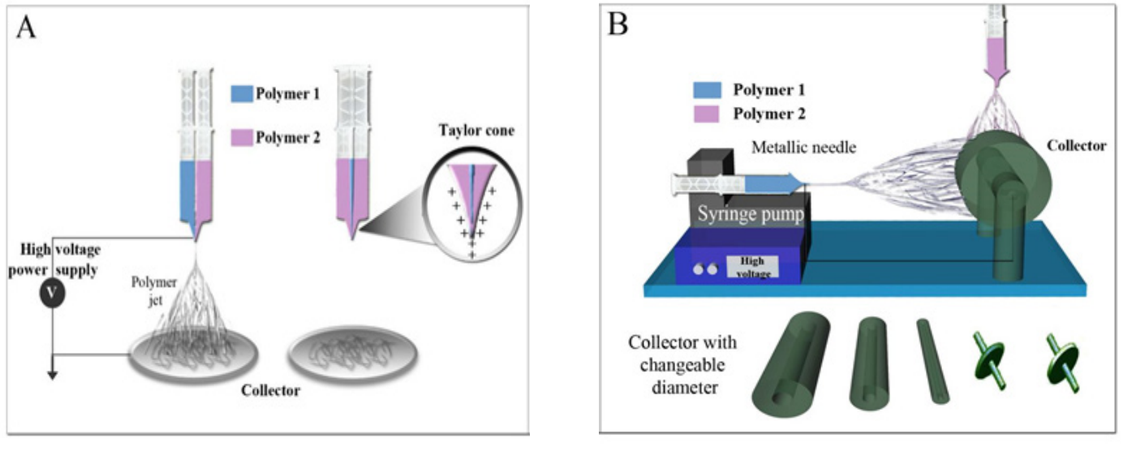

Figure 2 - (A) Schematic of side-by-side nozzle configuration. Two capillaries containing different polymer solutions are set side-by-side. As long as the two solutions have similar conductivities, a single Taylor cone will be developed, and a fiber jet containing both polymers will be produced. However, the relative amounts of each polymer can vary along the electrospun fiber. Also, schematic of coaxial nozzle configuration. A smaller capillary is set inside a larger capillary such that the long axes of the capillaries coincide. Different polymer solutions are passed through each capillary. At the tip of the capillaries the Taylor cone is formed and leads to the formation of fibers in which one polymer fiber is encapsulated within another (known as a core-shell morphology). (B) The rotational collector can be used with a unique polymer solution, but also permits to have two (or more) nozzles with different polymer solutions.

as well as the possibility of encapsulating different types of molecules (drugs, biomolecules, etc.). For example, the development of compliant composite drug eluting athrombogenic vascular graft with capacities of recruiting cells *in vivo* is one of the most promising approaches in vascular tissue engineering. Rolling, stacking and folding including cell-driven self-folding as a concept of 4D bioprinting (Li *et al.*, 2016; An *et al.*, 2016; Gao *et al.*, 2016) of electrospun matrices offer interesting opportunities for designing and engineering tissue engineering constructs of complex geometry (Cheng *et al.*, 2017, Mokhena and Luyt, 2017).

3. Hybrid fused deposition modeling and electrospinning nanofibrous technology

Combination of electrospinning with fused deposition modeling (FDM) opens a new opportunity for designing dual scale scaffold suitable for biofabrication of 3D tissue and organs (Fig. 3). Electrospinning dues to the biomimetic character of nanofibers provide superior matrices for cell attachment and biocompatibility, but material properties of electrospun matrices are inferior, and capacities for engineering 3D tissue constructs are limited. FDM allows fabricating 3D tissue engineered scaffolds of desirable patient-specific 3D shape and provides superior biomimetic material properties and better mechanical properties, but due to the large size of pores, the cell seeding is usually not optimal or time-consuming. Hybrid FDM and electrospinning dual scale scaffold technology eliminates limitation of both technologies and provides desirable synergistic beneficial effect (Park *et al.*, 2008).

One of the possible applications of hybrid technology is the fabrication of an auricular implant with optimal biomimetic material properties and enhanced biocompatibility. Patient specific computer-aided design of, for example, an auricular implant could be developed based on using laser scanning of the external ear (Wen *et al.*, 2008) and customized auricular implant could be fabricated by additive manufacturing technology such as a FDM and electrospinning will enhance the biocompatibility of the

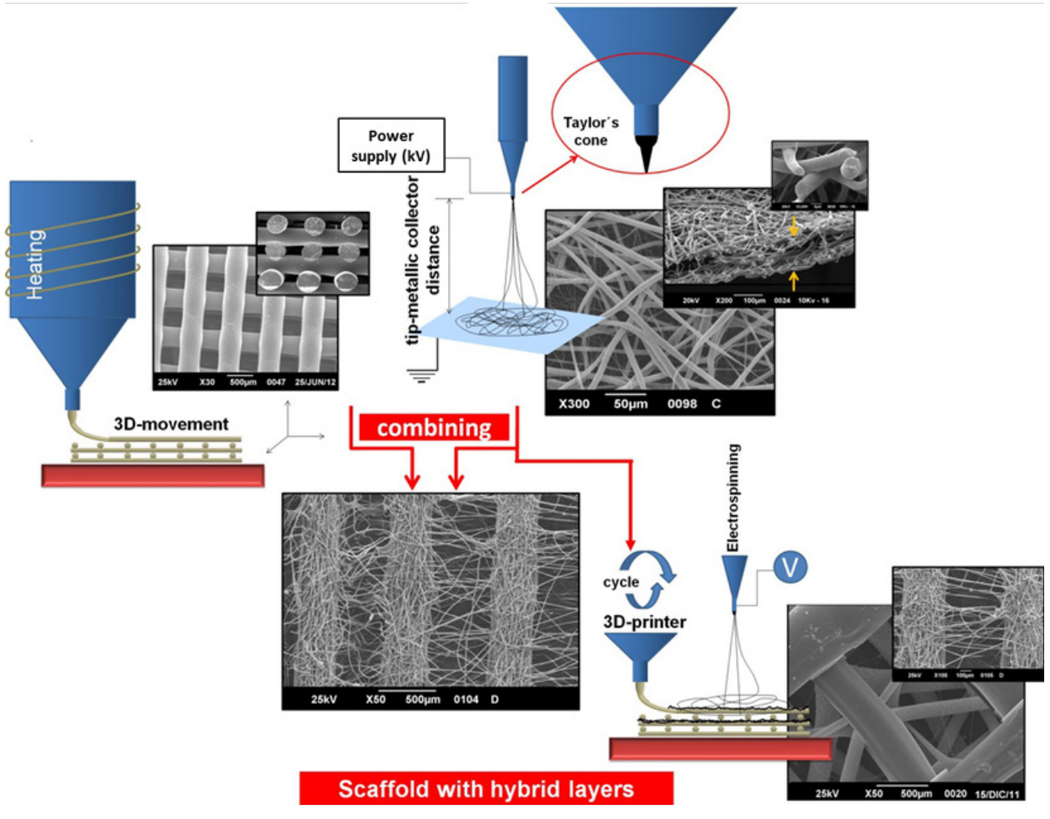

Figure 3 - Proposal of 3D dual scale polymer scaffolds (or hybrid scaffolds) produced by FDM 3D additive manufacturing technology and electrospinning (SEM micrographs supplied by B5IDA research group, Universidad Simon Bolivar, Venezuela) (Figure adapted from Sabino *et al.*, 2017).

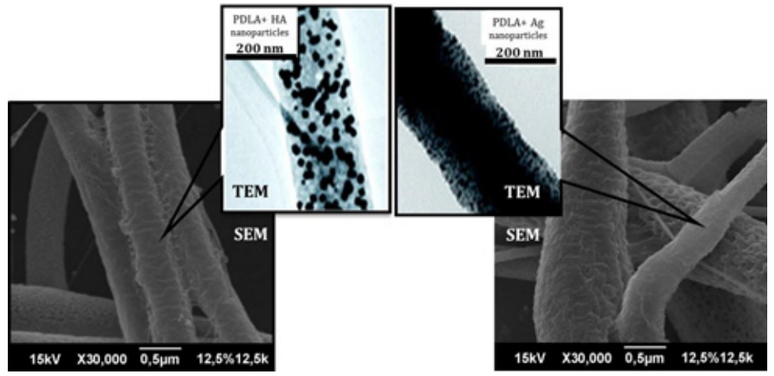

Figure 5 - Micrographs from Scanning and Transmission Electron Microscopy (SEM and TEM) of micro/nanofibers of PDLA (polymer D-lactic acid) charge with nanoparticles of hydroxyapatite (HA) and silver (Ag), obtained using electrospinning (images supplied by B5IDA research group, Universidad Simon Bolivar, Venezuela).

4. Conclusion and Future Perspectives

Nanotechnology applied in the tissue engineering field is rapidly evolving an ongoing process. As we tried to illustrate in this review paper, there is also strong potential for successful application of nanotechnology in human organ biofabrication which has macrolevel organization.

Nevertheless, the researchers on the intersection between the nanotechnology and the tissue engineering are still encountering some serious barriers. The first and most evident one is the toxicology of the nanomaterials.

Thus, the biocompatibility of nanomaterials for tissue engineering application must be seriously taken into account. The second challenge concerns the functionalization of the nanomaterials. Recent progress in designing drug-eluting nanofibers using coaxial electrospinning is a suitable example of evolution.

The third challenge is related to the design, synthesis and production of novel nanomaterials with biomimetic aspects. This trend is already evident in the fabrication of nanofibers using electrospinning technology. The development of composite nanomaterials is one of the most promising approaches in this direction.

The fourth challenge is the standardization of nanomaterials. In a wider view, to the achievement of the product certification, it is extremely necessary the standardization on certain stage of development of any emerging technology.

In the future, perspective functional biomimetic nanomaterials can enable regenerative medicine that means healing from inside and therefore decrease the necessity for tissue engineering *ex vivo*. Drug-eluting electrospun vascular graft is one possible example of this tendency.

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References

1. Aguirre-Chagala YE, Altuzar V, León-Sarabia E, Tinoco-Magaña JC, Yañez-Limón JM, Mendoza-Barrera C, Physicochemical properties of polycaprolactone/collagen/elastin nanofibers fabricated by electrospinning. *Materials Science and Engineering: C*; 76: 897-907 (2017).
2. An J, Chua C K, Mironov V, A Perspective on 3D Bioprinting. *International Journal of Bioprinting*, V.2, n.1 (2016).
3. Balogh A, Cselkó R, Démuth B, Verreck G, Mensch J, Marosi G, Nag ZK, Alternating current electrospinning for preparation of fibrous drug delivery systems. *International Journal of Pharmaceutics*, 495(1): 75-80 (2015).
4. Boland ED, Matthews JA, Pawlowski KJ, Simpson DG, Wnek GE, Bowlin GL,, Electrospinning collagen and elastin: preliminary vascular tissue engineering. *Front Biosci.;* 9: 1422-1432 (2004).
5. Bondar B, Fuchs S, Motta A, Migliaresi C, Kirkpatrick CJ, Functionality of endothelial cells on silk fibroin nets: comparative study of micro- and nanometric fibre size. *Biomaterials;* 29(5): 561-572 (2008).
6. Braghirolli DI, Steffens D, Pranke P, Electrospinning for regenerative medicine: a review of the main topics. *Drug Discovery Today* 19 (6), 743-753 (2014).
7. Cheng J, Jun Y, Qin J, Lee S-H, Electrospinning versus microfluidic spinning of functional fibers for biomedical applications. *Biomaterials*; 114: 121-143 (2017).
8. Gao B, Yang Q, 4D Bioprinting for Biomedical Applications. *Trends in Biotechnology*, V.34, Issue 9, pp746-756 (2016).
9. Groll J et al. Biofabrication: reappraising the definition of an evolving field. *Biofabrication*, V.8, n.1 (2016).
10. Hidalgo IA, Sojo F, Arvelo F, Sabino MA, Functional electrospun Poly(lactic acid) scaffolds for biomedical applications. Experimental conditions, degradation and biocompatibility study. *J. Molecular & Cellular Biomechanics*; 10(2): 85-105 (2013).
11. Hinton TJ, Lee A, Feinberg AW, 3D bioprinting from the micrometer to millimeter length scales: Size does matter. *Current Opinion in Biomedical Engineering*; 1: 31-37 (2017).
12. Innocente F, Mandracchia D, Pektok E, Nottelet B, Tille JC,

de Valence S, Faggian G, Mazzucco A, Kalangos A, Gurny R, Moeller M, Walpoth BH,Paclitaxel-eluting biodegradable synthetic vascular prostheses: a step towards reduction of neointima formation, *Circulation*; 120(11 Suppl): S37-45 (2009).

13. Kamoun EA, Kenawy ERS, Chen X A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. *Journal of Advanced Research*; 8(3): 217-233 (2017).
14. Lee CH, Cook JL, Mendelson A, Moioli EK, Yao H, Mao JJ, Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet.;* 376(9739): 440-448 (2010).
15. Li Y-C, Zhang YS, Akpek, A, Shin SR, Khademhosseini A, 4D bioprinting: the next-generation technology for biofabrication enabled by stimuli-responsive materials. *Biofabrication*, V.9, n.1 (2016).
16. Ma P X, Biomimetic materials for tissue engineering. *Advanced Drug Delivery Reviews*; 60: 184-198 (2008).
17. Mironov V, Kasyanov V, Markwald R, Nanotechnology in vascular tissue engineering: from nanoscaffolding towards rapid vessel biofabrication. *Trends in Biotechnology*; 26(6): 338–344 (2008a).
18. Mironov V, Kasyanov V, Drake C, Markwald RR, Organ printing: promises and challenges. *Regenerative Med*.; 3(1): 93-103 (2008b).
19. Mironov V, Kasyanov V, Markwald RR, Nanotechnology in vascular tissue engineering: from nanoscaffolding towards rapid vessel biofabrication. *Trends Biotechnol.;* 26(6): 338-344 (2008c).
20. Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR, Organ printing: tissue spheroids as building blocks. *Biomaterials*; 30(12): 2164-2174 (2009a).
21. Mironov V, Trusk T, Kasyanov V, Little S, Swaja R, Markwald R, Biofabrication: a 21st century manufacturing paradigm, *Biofabrication*; 1(2): 022001 (2009b). doi: 10.1088/1758-5082/1/2/022001
22. Mokhena TC, Luyt AS, Electrospun alginate nanofibres impregnated with silver nanoparticles: Preparation, morphology and antibacterial properties. *Carbohydrate Polymers*; 165: 304-312 (2017).
23. Murugan R, Ramakrishna S, Nano-featured scaffolds for tissue engineering: a review of spinning methodologies. *Tissue Eng.;* 12(3): 435-447 (2006).
24. Murugan R, Huang ZM, Yang F, Ramakrishna S, Nanofibrous scaffold engineering using electrospinning. *J Nanosci Nanotechnol.;* 7(12): 4595-4603 (2007a).
25. Murugan R, Ramakrishna S, Design strategies of tissue engineering scaffolds with controlled fiber orientation. *Tissue Eng*.; 13(8): 1845-1866 (2007b).
26. Park SH, Kim TG, Kim HC, Yang DY, Park TG, Development of dual scale scaffolds via direct polymer melt deposition and electrospinning for applications in tissue regeneration. *Acta Biomaterialia*; 4(5): 1198-1207 (2008).
27. Perán M, García MA, López-Ruiz E, Bustamante M, Jiménez G, Madeddu R, Marchal JA, Functionalized nanostructures with application in regenerative medicine. *Int J Mol Sci.;* 13(3): 3847-3886 (2012).
28. Rezende R, Azevedo F, Pedreira F, Kasyanov V, Wen X, Da Silva J, Mironov V, Nanotechnological Strategies for Biofabrication of Human Organs. *Journal of Nanotechnology*; Vol 2012 (on line):1-10 (2012a). doi:10.1155/2012/149264.
29. Roco MC, Nanotechnology: convergence with modern biology and medicine. *Curr Opin Biotechnol*.; 14(3): 337-346 (2003).
30. Sabino MA, Loaiza M, Dernowsek J, Rezende R, Silva JVL, Review: Techniques for manufacturing polymer scaffolds with potential applications in Tissue Engineering. *Revista Latinoamerica de Metalurgia y Materiales RLMM*.; 37(2): 1-27 (2017).
31. Stankus JJ, Guan J, Fujimoto K, Wagner WR, Microintegrating smooth muscle cells into a biodegradable, elastomeric fiber matrix. *Biomaterials*; 27(5): 735-744 (2006).
32. Takanari K, Hashizume R, Hong Y, Amoroso NJ, Yoshizumi T, Gharaibeh B, Yoshida O, Nonaka K, Sato H, Huard J, Wagner WR, Skeletal muscle derived stem cells microintegrated into a biodegradable elastomer for reconstruction of the abdominal wall. *Biomaterials; 113: 31-41 (2017).*
33. Wen Z Lin F, Shi T, Zhang R, Nian Y, Ruan J, Zh T, Fused deposition modelling of an auricle framework for microtia reconstruction based on CT images, *Rapid Prototyping Journal*; 14 (5): 280–284 (2008).
34. Wu W, Allen RA, Wang Y, Fast-degrading elastomer enables rapid remodeling of a cell-free synthetic graft into a neoartery. *Nat Med.;* 18(7): 1148-1153 (2012).
35. Zhan Q, Shen B, Fang Y, Deng X, Chen H, Jin J, Peng C, Li H, Drug-eluting scaffold inhibited in vivo pancreatic tumorigenesis by engaging murine $CCR4^{+}CD8^{+}$ T cells. *Colloids and Surfaces B: Biointerfaces*; 158: 469-473 (2017).
36. Zhu Te, Kui Yu M, Bhutto A, Guo X, Shen W, Wang J, Chen W, El-Hamshary H, Al-Deyab SS, Mo X, Synthesis of RGD-peptide modified poly(ester-urethane) urea electrospun nanofibers as a potential application for vascular tissue engineering. *Chemical Engineering Journal*; 315: 177-190 (2017).