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

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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 scaffolds 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; Guillenot 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 redefined and reaffirmed 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; Riczende et al., 2012; Perin et al., 2012). A quick systemic analysis consult on 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 synthetic) until composites mixing these groups. Looking at the biofabrication aspects ceramics and mainly polymers are highly used. On hybrid processes involving a 3D bio-printer and electrospinning as main topics in this paper, in general, the most common materials applied are alginate, collagen, chitosan, starches as being the natural ones plus PCL (poly caprolactone), PLA (poly lactic acid), PLGA (poly lactic-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 create three-dimensional structures that have a macroscale.

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; Braghinetti 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 polymers, 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 meshes 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 electrospin 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 (Iinocente 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 BSIDA research group, Universidad Simon Bolivar, Venezuela).

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 atherothrombogenic 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; Guo 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 due to the biomimetic character of nanofibers provide superior matrices for cell attachment and biocompatibility, but material properties of electrosprin 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 BSIDA research group, UniversidadSimon Bolívar, 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 (polyester D-lactic acid) charge with nanoparticles of hydroxyapatite (HA) and silver (Ag), obtained using electrospinning (images supplied by BSIDA 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 nanofibers 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 electros spun vascular graft is one possible example of this tendency.

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