Editorial

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Nanomedicine

2D imprinted substrates and 3D electrospun scaffolds revolutionize biomedicine

“Imprinting lithography and electrospinning are due to transform healthcare.”

First draft submitted: 16 March 2016; Accepted for publication: 22 March 2016; Published online: 13 April 2016

Keywords: cell response • electrospinning • imprinting • nano–bio interface • tissue response • topography

Momentous advances in engineering in the 1980s and 1990s reduced significantly the manufacturing cost of equipment that can produce nano to low microscale materials. This was a key milestone in the field of biomedicine, as from the early 2000s, we entered the era of nanobiomedicine. Essentially, with very low capital cost, every laboratory in the world could develop cell culture substrates and/or implantable devices with morphological features down to the nanometer scale. The rationale of using such small dimensional features derives from the fact that the extracellular space is comprised of nano- to microscale supramolecular assemblies. Further, cells employ probing filopodia extensions and contractile intracellular machinery to gather topographical, spatial, mechanical and chemical information from the extracellular environment, which consequently determines cell functionality, lineage commitment and fate. Among the various fabrication technologies, lithography constitutes the most popular 2D top-down method, while electrospinning represents the most widely used 3D bottom-up method [1–7]. The produced 2D and 3D constructs have enabled the study of cell and tissue responses at previously economical and technological prohibiting small scales and the rational design thereof for a specific application/clinical indication. Key to the design tenant is that topographical features and morphological structures should promote cellular polarization and organized extracel-

lular matrix assembly, resembling the natural conformation of tissues.

Using photolithography back in 1983, microscale V-shape grooves were created to study human gingival and porcine epithelial cell response [8]. By 1997, we were in a position to fabricate substrates with feature (square) accuracy down to 14 nm (depth) to study *Xenopus* neurites outgrowth using electron-beam lithography [9,10]. To date, we have successfully imprinted various gratings and geometric shapes along different scales onto thermoplastic polymers to create biologically relevant topographical interfaces. Data obtained from these and other studies clearly illustrate that topographical features are powerful modulators of cell morphology and differential function [11–15]. Further, nano- and microscale arrays have been shown to be potent tools in maintaining the phenotype fidelity of permanently differentiated cells and in differentiating stem cells toward specific lineages [16,17].

Multiple topographic arrays have also been used as high-throughput systems to elucidate the cell–surface interactions and enable identification of improved surfaces for optimal/required cell response [18,19]. Specifically, topographical features have been shown to directly modulate cell–material interaction and to affect the composition, orientation and conformation of adsorbed extracellular matrix components [20,21]. Further, nanoimprinted materials have been shown to either

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directly or indirectly influence the formation and maturation of focal adhesion complexes [22], a process though to modulate transcriptional events through adhesion-dependent phosphorylation of downstream signaling molecules (e.g., mediated focal adhesion kinases).

Shape memory polymers have also been investigated as responsive nanoscale materials, which can be switched, for example, from a surface, which presents an anisotropic topography to a planar surface with associated loss of cell orientation [23]. Furthermore, analogous systems studying grooves with switchable widths have been employed to apply mechanical force to regulate the shape and the cytoskeletal arrangement of rat stem cells, inducing lineage-specific differentiation of stem cell toward myogenic lineages in the absence of induction factors [24]. It can be inferred that nanoimprinted topographies that display a dynamic response to environmental changes may promote the development of tunable tissue-specific topography and play important future roles in smart, tissue engineered implants or lab-on-chip devices.

Unfortunately, 2D nano to low microimprinted substrates have failed to induce a therapeutic effect *in vivo* [25,26], which imposes the need for the development of nanotextured 3D scaffold fabrication technologies, such as electrospinning, for implantation purposes. Electrospinning was first appeared in the biomaterials field in 2001 [27,28]. Since then, it has been widely used in every single aspect of biomedicine. Numerous natural and synthetic polymers have been electrospun, although the harsh solvents are associated with denaturation of collagen [29,30]. Even stimuli-responsive polymers have been electrospun for various biomedical applications, including the production of living cell layers [31,32]. Advances in the fabrication process, such as rotating [33–35] or controlled porosity [36–38] collectors have enabled the production of bidirectionally aligned electrospun meshes or meshes with precise architectural features, respectively. Melt electrospinning, a combination of electrospinning and direct writing additive manufacturing, has given rise to a new family of scaffolds with more precise architectural features [39,40]. Blending, multilayering, surface treatment, dual electrospinning, coaxial electrospinning and electrospinning-co-electrospraying are a few of the developed technologies that have allowed sustained and localized delivery of bioactive/therapeutics

molecules at the side of interest [41–43]. Electrospinning-co-electrospraying and coaxial electrospinning have also been used to deposit living cells onto the produced scaffolds [44–47]. With respect to cell response, electrospun mats, alone or in combination with biochemical/biological cues, have been shown to maintain phenotype fidelity of permanently differentiated cells and/or to direct stem cell lineage commitment [48–54]. In preclinical setting, electrospun scaffolds, alone or with bioactive/therapeutic molecules and various viable cell populations have been implanted literally to every single preclinical model known, and the results in all instances have been very promising (301 papers; source: PubMed; term searched: ‘electrospinning’ and ‘*in vivo*’ in title/abstract). Despite this *in vivo* success in preclinical model, to date only three clinical trials are registered (source: ClinicalTrials.gov; term searched: ‘electrospinning’). This is not surprising, considering that electrospinning is only 15 years old.

Overall, we believe that the full potential of imprinting and electrospinning has yet to be realized. Cell-based therapies require *in vitro* expansion of cells to obtain therapeutically relevant cell numbers with preserved native phenotype. Given that customarily used tissue culture plastics fail to imitate the native *in vivo* milieu, 2D imprinted materials with optimal/physiological surface topography and substrate rigidity are expected to be key players in the field of cell therapies to optimally propagate cells *in vitro*, prior to implantation. In the years to come, the manufacturing cost of 3D electrospun scaffolds is expected to be reduced. As such, we foresee that more and more electrospun materials will reach the clinical setting. In our view, imprinting lithography and electrospinning are due to transform healthcare.

Financial & competing interests disclosure

This publication is supported by the Health Research Board (grant agreement number: HRA_POR/2011/84) and the Science Foundation Ireland and the European Regional Development Fund (grant agreement number: 13/RC/2073). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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