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Electrospinning and microfluidics: an integrated approach for tissue engineering and cancer

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Progress in microfluidic technology has enabled precise manipulation of small volumes of fluids, leading to the development of low-cost and portable systems that have shown considerable promise in biomedicine. Although these functional devices have gained a great deal of attention over the past decades, a new fascinating trend concerns the integration of microfluidics with other fabrication techniques, with particular regard to electrospinning and additive manufacturing.

In this chapter, the attention will be focused on integrative approaches obtained combining microfluidics and electrospinning, highlighting the recent advances and challenges in the tissue engineering framework. Indeed, although this innovative trend is still at its beginning, significant results have been achieved both in the microfluidic-aided fabrication of novel microstructured materials and in the development of new biosensors and analytical devices for point-of-care diagnostics.

8.1 Electrospinning: an overview

The origin of electrospinning (ES) as a viable fiber spinning technique can be traced back to the first years of the 20th century. In 1902, J. F. Cooley [1] deposited a patent that disclosed the principle and system to generate powders or fibers using a high-voltage apparatus. From that pioneering work, the technique has been further developed and refined, as documented by many articles elucidating the history of electrospinning [2–4], for example, the patents deposited in the 1930s by Formhals [5,6]. However, since the first appearance of this technique, a long time passed before a theoretical study on the jet forming process was made [7,8]; the characteristic conical shape assumed by the drop was later referred to as the Taylor cone, using the name of its discoverer.

The use of the word *electrospinning* is quite recent; it was introduced for the first time in 1995 in two different scientific articles by R. H. Reneker and coworkers [9,10]. The mechanism of fiber production is relatively easy and can be summarized as follows. A high-voltage (generally in the order of tens of kV) is applied between an extrusion system (i.e., nozzle) and a collector. A polymeric solution is then fed at a constant flow rate to the nozzle where it starts elongating forming the Taylor cone. The initial

Electrofluidodynamic Technologies (EFDTs) for Biomaterials and Medical Devices.
https://doi.org/10.1016/B978-0-08-101745-6.00008-6

ejection from the cone undergoes a chaotic motion and, as the jet travels through the atmosphere, the solvent evaporates leaving behind polymer fibers that are deposited on the collector [11].

Being an easy, unique approach in which electrostatic forces are used to produce fine fibers, electrospinning has attracted many researchers driven by the possibility to fabricate fibers with both micro- and nanostructural characteristics enabling the development of advanced materials to be used in sophisticated applications. Moreover, an appealing aspect of electrospinning is the high level of fine-tuning granted by the many process parameters that can be modified and that greatly affect the final product. In fact, changes to the final fiber morphology and surface topography and, consequently, characteristics and properties of the electrospun mat, are influenced not only by the processing parameters (e.g., solution feed rate, applied voltage, nozzle-collector distance) and spinning environment, such as humidity and temperature, but also by the material properties, like solution rheology, concentration, viscosity, surface tension, conductivity, and solvent vapor pressure. In this regard, Seyedmahmoud et al. showed the use of statistical methodologies to gain a quantitative and systematic insight about the effects and interactions between the main process parameters and scaffold properties [12,13]. Thus, the electrospinning process, despite the apparent simplicity, can fabricate complex electrospun fibers whose characteristics and properties, morphology, and microstructure are affected by a high number of parameters.

Generally, electrospun structures are characterized by high surface-to-volume ratio, high porosity, dimensions ranging from nano- to microscale, and variable pore-size distribution, showing morphological similarities to the natural extracellular matrix (ECM). Thus, electrospun fibers have been proposed for the fabrication of tissue engineering scaffolds [14–18].

Moreover, electrospinning technique is also easily adjusted to allow fiber functionalization by the addition of molecules and/or nanoparticles. This, can be achieved in different ways. For example, nanoparticles can be embedded in the precursor solution in a “one-pot route” [19,20] or following electrospinning [21], by immersing electrospun structures into colloidal solutions. New trends also entail the functionalization of the electrospun nanofibers by a more refined modification of their surfaces to further enhance the material performance. As an example, in the fields of neural and cardiac tissue engineering, the topology of the electrospun nanofibers was modified to super porous or grid shaped [22–24]; also, in order to guide the growth, apoptosis, and differentiation of cells, nanofibers have been coated or modified with drugs and DNA [25–30]. Moreover, another great advantage of electrospun fibers over nanomaterials obtained with “classical” methods is the possibility to control the overall alignment along desired directions or to obtain organized fiber patterns through the use of optimized metal collectors [31]. Thanks to all those advantages, and especially with regard to the high porosity, large specific surface area, and wide material selection, electrospun fibers have been widely utilized in many fields related to biomedicine as tissue engineering, regenerative medicine, and drug delivery devices [32].

To summarize, electrospinning has the advantages of simplicity, efficiency, low cost, high yield, and high degree of reproducibility, as well as high versatility in the obtained compositions and architectures. However, to successfully mimic the

complexity of the in vivo environment, it might be necessary to combine electrospinning with other fabrication techniques [32–34].

8.2 Microfluidics in biomedical research

Microfluidics is a relatively new research field that deals with the manipulation of fluids at the microscale, allowing their precise control and processing [35]. Flow in microfluidic regime shows effects that can hardly be seen at a greater scale. In particular, the viscous and capillary forces become dominant with respect to the inertial ones; as a result, fluids are generally characterized by laminar flows with very low Reynolds number ($Re << 1$) [36].

Since the 1990s, microfluidics has grown enormously, becoming a first-choice approach for an increasing number of applications in a broad set of research fields such as analytical chemistry [37,38], bioassays [39,40], materials science [41,42], biology [43,44], optics [45], etc. Independently from the application, the main advantages of microfluidics are: (1) the extremely reduced volume of reagents/samples needed to run the experiments, (2) the high repeatability of the experiments performed in the microchannels, (3) the ease of device design and fabrication, (4) the low fabrication cost per unit, and (5) the possibility to integrate fluidic units such as mixers, valves, pumps, etc. within the devices achieving an extremely high degree of flow control.

In the last decade, microfluidics has been extensively studied to address relevant questions and problems in biomedical research [46]. The plethora of applications developed so far can be classified into four main categories:

1. Microfluidic devices designed to carry out specific analyses of biologically important molecules, generally referred as a micro total analysis system (μ-TAS);
2. Microfluidic devices specifically designed for biological purposes such as cell culture, cell–cell interaction, cell sorting, etc.;
3. Microfluidic chips devised for the fabrication of functional microtissues/organs to be used as predictive in vitro models (referred to as organ-on-a-chip devices);
4. Microfluidic devices for the fabrication of tailored biomaterials to be used as scaffolds or cell carriers for tissue engineering applications.

μ-TASs have been proposed since the beginning of 1990s [47]. These systems, when fully developed, contain elements for the acquisition, pretreatment, separation, posttreatment, and detection of a variety of samples. All these steps are achieved by the integration of functional modules, which can include, for instance, specific channel networks for the creation of gradients in the analyzed flow [48], integrated optics for the excitation and detection of fluorescently labeled molecules or cells [49], DNA amplification by polymerase chain reaction [50], etc. So far, μ-TASs have found several applications in genomics [51], proteomics [52], clinical diagnostics [53], drug discovery [54], and biosensors [55].

Microfluidic devices represent a suitable platform for cell handling and culture. In fact, the possibility to study specific biological phenomena in tailored microenvironments at the same scale length of cells has prompted engineers and biologists to develop numerous applications aimed at investigating cell polarization [56] and cross-talk

between different cell types [57,58], cell response to specific active compounds, biomolecules, or nanoparticles [59,60], etc. These studies are extremely interesting as the results can be better and more reliably analyzed if compared to population-level studies in which the results are always averaged on the whole cell population.

Besides these studies, an enormous wave of excitement has spread in the tissue engineering community for the development of new cell culture platforms, the so-called organs-on-chips. An organ-on-a-chip is a microfluidic cell culture device created via soft-lithography and replica molding that contains continuously perfused and/or actuated (generally vacuum-actuated) microchambers/channels inhabited by living cells arranged to simulate tissue- and organ-level physiology [61]. By recapitulating the multicellular architectures, tissue–tissue interfaces, physicochemical microenvironments, and vascular perfusion of biological systems, these devices produce unprecedented levels of tissue and organ functionality not possible with conventional 2D- or 3D-culture systems [62]. They also enable high-resolution, real-time imaging and in vitro analysis of biochemical, genetic, and metabolic activities of living cells in a functional tissue and organ context. So far, researchers have fabricated chips to study several organs such as liver, kidney, lung, intestine, heart, fat, and skin demonstrating that organs-on-chips can mimic specific organ-level functions [63,64].

The intrinsic advantages of microfluidic technology in handling and processing fluids at the microscale also have been exploited for the fabrication of tailored biomaterials for tissue engineering applications. An abundant number of microfluidic systems have been proposed for the fabrication of microparticles and microcapsules by using both synthetic and natural polymers [41,65]. The obtained products are extremely monodisperse (CV < 5%), they can be produced in rather wide ranges of sizes and shapes (from few tens to hundreds of micrometers in diameter), and they can encapsulate living cells or active molecules for drug-delivery applications.

Furthermore, multiphasic systems have been proposed for the fabrication of either monodisperse porous scaffolds or porous microparticles [66]. These materials have been shown to have enhanced permeability properties and cell colonization after seeding when compared to conventional porous scaffolds [67]. In other applications, researchers have used microfluidic systems for the production of microfibers and nanofibers demonstrating that it is possible to tailor their morphology and improve their production rate [68–71]. Among them, graded electrospinning nanofibers have been successfully developed through a microfluidic-assisted ES approach [32]. This system, enabling accurate and tunable mixing of the precursor solutions with variable nanoparticles and biomolecule concentrations before the electrospinning process, produced nanofibers with spatially controlled gradients and enhanced functionality.

8.3 Hybrid systems

Despite their growing diffusion, microfluidic chips still face many problems in real-world applications since most of them are 2D systems, thus, recapitulating only partially the real in vivo environment experienced by cells [72]. To overcome this issue, various membranes, such as polydimethylsiloxane (PDMS) [64], polycarbonate (PC)

[73,74], and/or 3D-hydrogel matrices [72,75,76] have been successfully integrated into microfluidic devices.

Among the others, electrospun membranes have been demonstrated to be a promising substrate for integration into microfluidic devices [77] due to their straightforward fabrication procedure and relatively large specific surface area. However, electrospun membranes are often too fragile and easily crinkled for typical sealing methodologies, and the roughness of the surface further contributes to the reduction of the bonding efficiency. In addition, considering that PDMS can form tight, irreversible seals only on a very limited number of materials, novel methods have been developed and optimized prior to successfully entrapping electrospun membranes into soft-lithographically obtained microfluidic chips.

In early studies, large electrospun fiber mats have been pressed and held in place by the bonded PDMS and the glass slide or sealed by using adhesive tape [77–79]. Although press fitting can generate tight seals, the hydraulic pressure that can be applied in such a system is lower compared with irreversibly bonded PDMS microfluidic networks. Furthermore, if electrospun fibers are located both inside the channel and at the PDMS glass interface, small amounts of liquid could easily creep over the channel boundaries [80]. Thus, more innovative solutions have been developed to integrate spatially controlled electrospun fibers within microfluidics PDMS devices [80,81]. Among the others, a simple and versatile electrolyte-assisted electrospinning process has been engineered for the fabrication of a free-standing nanofiber membrane on complex-shaped PDMS microcavities, using an electrolyte solution as a grounded collector instead of a conventional metal collector [81].

As a result of these bonding optimization protocols, several studies have reported on the combined use of electrospun membranes and microfluidic devices for a wide range of applications, spanning from advanced tissue-engineered scaffolds to miniaturized bioanalytical systems for point-of-care diagnostic. This growing interest is shown by the increasing number of related publications during the last 15 years (Fig. 8.1).

A schematic overview of main application areas of these integrated approaches is reported in Fig. 8.2 together with some significant illustrative examples.

8.3.1 *Hybrid tissue-engineered* in vitro *models*

To explore the full potential of electrospun fiber substrates and to investigate cellular responses in greater detail into highly controlled microenvironments, several researchers have shown that the incorporation of electrospun fibrous mats into microfluidic chips could be beneficial for in vitro cell culture studies. For example, Lee et al. developed an integrated biomimetic system consisting of a PDMS-based microfluidic chip and a nanofiber polymer network, and evaluated the feasibility of human bone marrow–derived mesenchymal stem cell culture under different perfusion conditions and surface characteristics [82].

As a further improvement, patterned electrospun fibers have been produced and successfully integrated into microfluidic devices with the aim to better mimic the topography of the natural ECM [80,83,84]. This ability to create controlled cell

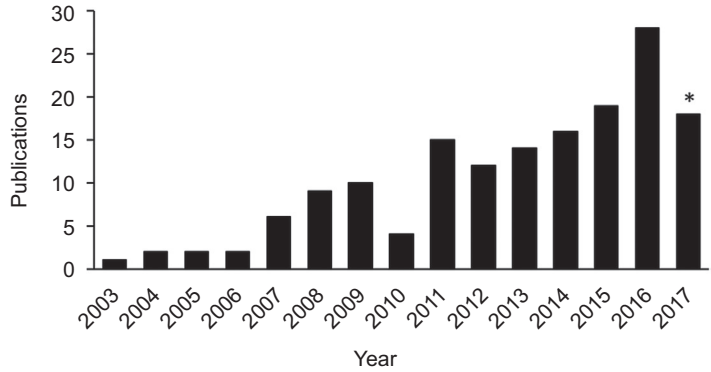

Figure 8.1 Number of publications on hybrid systems combining electrospinning and microfluidics during the last 15 years, (*until July 2017). Research items: "electrospinning" and "microfluidic".
From Scopus.

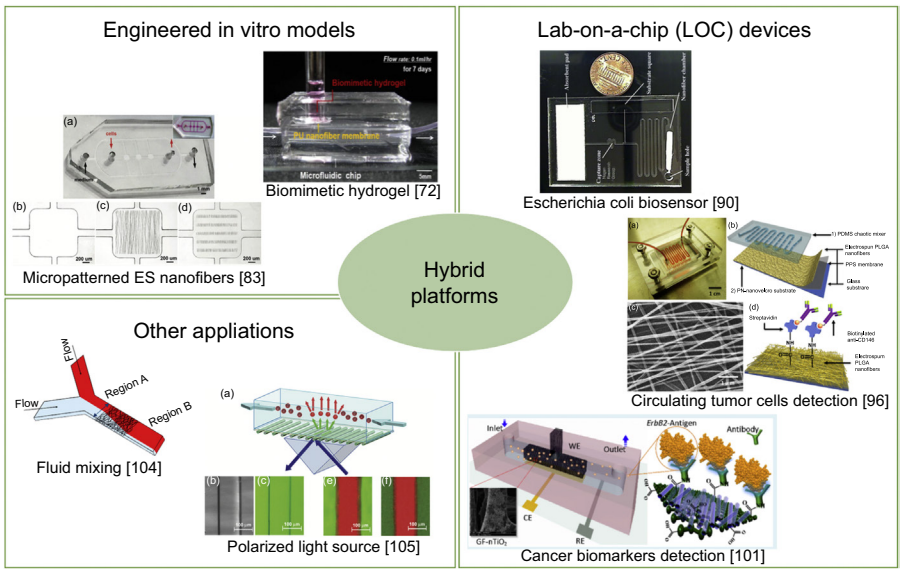

Figure 8.2 Schematic overview of main application areas of integrated approaches.

microenvironments through spatially defined fibrous structures becomes increasingly important for studying and/or controlling phenotype expression in tissue engineering and drug-discovery applications. In this framework, fiber pads have been integrated with microfluidic networks to study the combined effects of surface topography and chemical gradients on neural stem cells cultured in microfluidic channels on differently aligned ES substrates [80]. The combined effect of microfluidics and patterned-electrospun fibers on hepatocyte behavior has also been studied [84]. Hepatocytes cultured in a controlled system under an optimized flow exhibited

restored hepatocyte polarity and biliary excretion, and maintained liver-specific functions. In these conditions, they were able to produce sensitive and consistent Ag nanoparticles toxicity responses at different time points, demonstrating the feasibility of the patterned fiber-embedded microfluidic chips as a potential in vitro screening model for toxicological studies [84].

Alternatively, the combination of 3D-hydrogel and microfluidic devices also has been reported as a means to provide in vivo–like 3D conditions [75,76]. A leading example in this sense has been reported by Lee et al. who integrated an ECM-based biomimetic hydrogel into a microfluidic chip to monitor glioma cells' alignment and migration. As a further improvement, a polyurethane electrospun membrane has been integrated in the system, not only to prevent the collapse of the hydrogel into the microfluidic channel but also to act as a selective porous membrane to regulate the diffusion of media and growth factors into the hydrogel [72].

Although still in its infancy, the described approaches offer a large potential for the mimicking of in vitro cell microenvironment and represent a competitive strategy to study the interplay of flow conditions, surface properties, and soluble factors on cell behavior and cellular fate processes.

8.3.2 Hybrid lab-on-a-chip devices

Analytical assays in microfluidic regime have shown significant improvements over conventional bench-top assays across a range of performance metrics. Specific advances have been made in terms of reagent consumption, assay automation, and multiplexing capability [85]. These miniaturized bioanalytical systems have particularly benefited from the use of electrospun membranes thanks to their high specific surface area and macro/mesoporous structures that lead to high levels of protein absorbance.

Over the years, electrospun matrices have been successfully used to incorporate many biological species such as ECM proteins, growth factors, antibodies, and enzymes, with the aim to guide biological response, create nonspecific binding-resistant surfaces, and/or increase the stability of biological molecules. These biomolecules can be directly spun into fibers during electrospinning, coupled with secondary polymers that enhance their spinnability, or they can be immobilized on nanofibers postspinning. Regardless of the functionalization method, the high surface area of electrospun-nanofiber mats results in an increase of the immobilization efficiency when compared to conventional substrates [86].

Therefore, electrospun matrices have been recently adopted within bioanalytical microfluidic systems to enhance sample preparation (filtration, separation, and concentration) and analyte detection [87–89]. In some of these applications, water-soluble nanofibers have been used to facilitate on-chip reagent storage in microfluidic biosensors through the immobilization of biological molecules directly within fiber-spinning dopes [89,90]. As an example, a horseradish peroxidase-tagged antibody has been successfully encapsulated into water-soluble polyvinylpyrrolidone nanofibers, guaranteeing stable and long-term storage of the biorecognition element on-chip [90].

In alternative, non-water-soluble electrospun nanofibers have been variously functionalized and used as a substrate for microfluidic HIV immunoassays [78],

Escherichia coli detection [88], and on-chip sample concentrators [87]. To this aim, standard immobilization procedures have been purposely modified to functionalize nanofiber mats after being bonded into microfluidic channels [88,91]. In these applications, the non-water-soluble nanofibers could withstand the fluid flow within the channels, dramatically increasing the functional surface area available within the devices. In particular, Yang et al. reported the use of electrospun highly fibrous membranes as a protein-adsorption substrate in microfluidic immunoassays [78]. Resembling enzyme-linked immunosorbent assays (e.g., ELISA), microfluidic immunoassays involve the immobilization of the pathogenic antigen on a solid substrate. The substrate is then incubated with the serum that may contain the corresponding primary antibody, which can be captured by the immobilized antigen. A second labeled detection antibody is finally introduced to determine the amount of the primary antibody in the serum. According to this working principle, researchers created a PC nanofibrous membrane, sandwiched it between a glass substrate and a PDMS chip, and evaluated the performance of the membrane for promoting the adsorption of IgG and detecting HIV-specific antibodies from human serum samples. Compared with a commercial track-etched PC membrane having a uniform pore size, the synthetized nanofibers showed higher binding capacity and improved signal-to-noise ratio. Since polyvinylidene fluoride (PVDF) is a material commonly used for protein adsorption in Western blot analysis, similar results have been obtained embedding a PVDF nanofibrous membrane in a multichannel microfluidic assay [77]. The improved sensitivity of these microfluidic immunoassays can potentially facilitate the diagnosis of other diseases for which the detection is based on antigen/antibody recognition, such as hepatitis, many types of venereal diseases, severe acute respiratory syndrome, and the avian flu virus.

8.3.3 Hybrid microfluidic platforms for cancer research

Point-of-care detection devices are highly desirable for early diagnosis of diseases, but they require high sensitivity and specificity with minimum sample consumption and easy operation. Microfluidics holds great promise in this field, especially for cancer diagnosis. Developing and applying state-of-the-art microfluidic technologies to address the unmet challenges in cancer can expand the knowledge on biological mechanisms and improve the management of disease and patient care.

According to the scientific literature, four critical areas have been identified in cancer research in which microfluidics can change the current paradigm. These include: (1) cancer cell isolation, (2) molecular diagnostics, (3) tumor biology, and (4) high-throughput screening for therapeutics [92]. Concerning the first point, circulating tumor cells (CTCs) detection can be extremely valuable to cancer diagnosis in early stages and treatment choice. In this regard, one of the more active research groups is the team of Prof. Hsian-Rong at UCLA who developed a “NanoVelcro” cell-affinity substrate in which CTCs capture agent-coated nanostructured substrates have been utilized to immobilize CTCs with high efficiency. Based on the unique NanoVelcro working mechanism, the research team has produced and validated three generations of NanoVelcro chips capable of successfully detecting, isolating, and purifying CTCs from blood samples. First, they pioneered a unique NanoVelcro substrate, by using

3D-nanostructured elements—specifically, a silicon-nanopillar array—which allows for enhanced local topographic interactions with nanoscale components of the cellular surface (e.g., microvilli and filopodia) and results in vastly improved cell-capture affinity compared to flat Si substrates. Upon coating with epithelial-cell adhesion-molecule antibody, the system exhibited outstanding cell-capture efficiency in a stationary device setting [93]. Secondly, they further improved CTCs capture efficiency by integrating a lithographically patterned NanoVelcro substrate with an overlaid PDMS mixer to enhance contact frequency between flow-through CTCs and the substrate [94]. Finally, they introduced a next-generation NanoVelcro chip to replace the nontransparent silicon substrate with a transparent one, prepared by depositing electrospun PLGA nanofibers onto a commercial laser microdissection slide, capable not only of capturing CTCs with high efficiency but also enabling highly specific isolation of single CTCs immobilized on the nanosubstrate without contamination by white blood cells. In the presence of different capture agents, these NanoVelcro chips have been used to capture CTCs from several types of solid tumors, including prostate, breast, lung, and pancreatic cancer, as well as melanoma [95–97].

In addition to cellular approaches, other biomolecules can be monitored for cancer diagnosis such as circulating tumor DNA, microRNAs, proteins, and serum microvesicles [92,98,99]. Since microfluidics exhibits high sensitivity and accuracy for detecting cancer-specific biomarkers present at low concentrations [92], the quantification of protein biomarkers has also received some attention for the diagnosis and prognosis of cancer [100]. In this framework, a label-free microfluidic immunosensor with femtomolar sensitivity and high selectivity for early detection of epidermal growth factor receptor 2 (ErbB2, or HER2/neu) protein has been developed [101]. Among human epidermal growth factor receptor family, ErbB2 is worldwide recognized as a marker for early breast cancer diagnosis [102,103]. Thus, this sensor utilizes a uniquely structured immunoelectrode made of porous hierarchical graphene foam modified with electrospun carbon-doped titaniumdioxide nanofibers as an electrochemical working electrode. To detect interfacial changes originating from biorecognition events at the working electrode, electrochemical impedance spectroscopy and differential pulse voltammetry have been used [101].

Schematic illustrations of two representative examples of microfluidic platforms for cancer diagnosis can be found in Fig. 8.2.

8.3.4 Other applications

Although microfluidic networks have been shown to efficiently generate spatially and temporally defined liquid microenvironments, to date the effects of the electrospun-fiber mats on the fluid flow have been scarcely studied. In one of this few works, two different polymers, namely PVA and PS, have been used to produce fibers with different diameters and morphologies with the aim to study their effect on fluid mixing within microfluidic channels [104]. An appreciable passive mixing has been demonstrated for mats with more inhomogeneous morphology and smaller fiber diameters (450–550 nm). Additionally, passive mixing can be smartly coupled with the above reported use of electrospun substrates for analyte concentration, immobilization,

and/or detection; hence, microanalytical systems can take advantage of this dual functionality and avoid the use of additional mixing structures when already using nanofibers as functional components in their systems.

Furthermore, hybrid systems integrating electrospun membranes and microfluidic devices have gained interest also in optical analysis as a means to provide high sensitivity and short response time in lab-on-a-chip systems. In this context, polarized light-emitting electrospun nanofibers have been used as a nanoscale light source and integrated into optical sensing devices for lab-on-a-chip applications [105].

For the sake of completeness, although the research focus of this work is on integrated approaches for tissue engineering and cancer diagnosis, representative studies regarding the use of ES nanofibers as a catalyst in microfluidic fuel cells [106] or as a photocatalyst in novel microfluidic-based photocatalytic microreactors [107] should also be mentioned.

8.4 Conclusions

Electrospun nanofibers have been used with great success as tissue engineering scaffolds, in drug-delivery studies, and, more recently, as high-efficiency sensing platforms. At the same time, microfluidics and PDMS perfusion systems offer a very well-suited microenvironment for cell culture and enable reduced consumption of reagents.

The integration of these technologies has led to enormous advantages in several tissue engineering fields, as well as in the development of new biosensors and analytical devices for point-of-care diagnostics. In particular, the integration of electrospun membranes into microfluidic chips has provided a new means of fabricating complex tissue-engineered systems of considerable utility as in vitro models for toxicological studies.

On the other hand, miniaturized bioanalytical systems have particularly benefited from the use of electrospun nanofibers to enable further development of portable devices that require smaller reagent and sample volumes than traditional devices, making them more accessible for use in point-of-care settings, especially for early cancer diagnosis.

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