Figure 1.8 Representative SEM images of the distinct structures/morphologies: (a) β-PVDF film obtained by the doctor blade technique after mechanical stretching. Inset: The α-PVDF film before mechanical stretching. Scale bars: 5 μm (main) and 10 μm (inset). (b) β-PVDF film obtained by spin coating. Scale bar: 5 μm. (c) P(VDF-TrFE) film obtained by screen printing. Scale bar: 10 μm. (d) Porous P(VDF-HFP) film obtained by NIPS. Inset: Cross-section. Scale bars: 10 μm (main) and 100 μm (inset). (e) Porous P(VDF-CTFE) film obtained by NIPS. Inset: Cross-section. Scale bars: 30 μm (main) and 100 μm (inset). (f) Porous β-PVDF film obtained by NIPS (cross-section). Scale bar: 100 μm. (g) Porous β-PVDF film obtained by TIPS. Scale bar: 100 μm. (h) β-PVDF scaffolds obtained by solvent-casting particulate leaching. Scale bar: 500 μm. (i) β-PVDF scaffolds obtained by solvent casting and 3D nylon template. Scale bar: 400 μm. (j) β-PVDF scaffolds obtained by freeze extraction with a 3D PVA template (optical microscope image). Scale bar: 1 mm. (k) Patterned porous P(VDF-TrFE) structure obtained by replica molding. Inset: Cross-section. Scale bars: 500 μm. (l) Randomly electrospun β-PVDF fibers obtained by electrospinning. Inset: The oriented electrospun PVDF fibers. Scale bars: 10 μm. (m) β-PVDF spheres obtained by electrospraying. Scale bar: 5 μm. Source: Ribeiro et al. [147]/with permission of Springer Nature.

Surface charge and charge variation of piezoelectric and magnetoelectric biomaterials in bone, muscle, and nerve regeneration have been studied using scaffolds in film morphology [21, 112, 113, 132, 149, 150]. Besides, films with electroactive properties are designed for wound dressings due to their ability to adhere to the skin and protect the wound, as well as to be combined with different pharmaceutical compounds, including antibiotics, to promote wound regeneration and prevent bacterial infections [151–153].

1.5.2 Electrospun fibers

The fibrillar architecture of some extracellular matrix components, such as laminin, fibronectin, collagen, and elastin, has inspired the design of scaffolds with similar structures. Fiber scaffolds can be obtained by different methods and techniques, including phase separation, self-assembly, electrospinning, and melt electrowriting [154]. Electrospinning is the most used technique, due to its easy handling, low cost, and versatility. Through this technique, natural, synthetic, or composite polymer-based fibers can be obtained in random and oriented morphologies, with or without internal porosity, and with the most varied range of diameters, from micro- to nanofibers [155]. Because of fibers' easy processing and tailoring and large surface area-to-volume ratio, electrospun fibers are one of the most required and studied morphologies for TE applications. The electrospinning setup is composed of a syringe pump, a syringe filled with a polymeric solution and connected to a metallic needle, a metallic collector, and a power supply with high-voltage directly connected to the needle and the collector. Its functioning is based on an applied voltage to the polymeric solution, which is expelled as an electrically charged viscoelastic jet, with a controlled flow rate given by the syringe pump, toward the metallic collector. During this trajectory, the solvent evaporates from the jet, and the polymeric fibers are deposited onto the collector. Besides solution formulation, electrospinning

parameters can be optimized to tailor the obtained fibers, such as the flow rate, applied voltage, needle diameter, distance between the needle and the collector, and the type of collector used [147]. The use of a static collector allows to obtain fibers mats with random fiber distribution, and a rotative collector allows for obtaining aligned fiber morphologies.

Fibers not only mimic fibrous components of ECM but also enable the control of cell directionality by contact orientation, which is essential for specific applications. For instance, oriented fibers can be used to promote the directional growth of different cell types, such as neural [156, 157] and muscle cells [149, 158], to develop nerve guidance conduits to repair nerve defects [159, 160], and for the guidance of new bone formation [161, 162]. Electroactive fibers, either conductive, piezoelectric, or magnetoelectric composites, can deliver morphological and biophysical cues that are particularly interesting for TE [6, 163]. For instance, electrical stimuli given by fibrous scaffolds have proven to enhance cardiogenesis [164], myogenesis [165], neurite extension [166], and peripheral nerve repair [167].

1.5.3 3D Porous Scaffolds

The scaffold's internal structure, such as porosity, pore size, and interconnectivity, is highly influential in cell development. These features enable cell infiltration, migration, and interconnection, nutrients and waste diffusion, and help with tissue vascularization [168]. Porous architectures can be reproduced into films, fibers, membranes, or more complex 3D structures, using a variety of techniques, including phase separation, gas forming, salt leaching, and solvent-casting on 3D templates [147]. The porosity of the scaffolds is intimately related to its mechanical performance, for instance, higher porosities, in most cases, lead to decreased Young's modulus, but on the other side lead to an improved surface area that is available for cell attachment, so once again, the technique to be chosen is highly dependent on the application [169]. Porous scaffolds are often used for bone TE due to their resemblance with the trabecular bone [170, 171], but are also studied for other tissues such as neuronal [170, 171], muscle [172], and cardiovascular [173].

Because cells are surrounded by complex 3D microenvironments, porous 3D scaffolds are a relevant morphology for TE approaches [174]. These 3D structures can be obtained using the previously mentioned conventional methods but due to the scaffolds' complex 3D structure, these methods can be disadvantageous in terms of causing cytotoxicity, because the solvents are more difficult to completely evaporate during processing, and scaffolds' microstructure and resolution are more difficult to control [175]. As an alternative, rapid prototyping approaches can be used to develop 3D scaffolds. Rapid prototyping technologies, or additive manufacturing, include selective laser sintering, fused deposition molding, stereolithography, and 3D printing. These technologies are integrated with computer-aided design (CAD) software and scaffolds can be designed with controlled macro- (size and shape), micro- (pore size and shape, porosity, distribution, and porous interconnection), and nano-architecture (e.g., surface roughness and patterning). The controllable architecture of scaffold porosity allows for the

manipulation of cellular dynamics and the facilitation of cell attachment, elongation and proliferation, nutrient diffusion, and vascularization, with the potential to revolutionize TE and regenerative medicine by designing 3D scaffolds to meet the needs of individual patients in the scope of personalized medicine [176]. Porous scaffolds are being studied as delivery systems in addition to tissue repair and regeneration. Their interconnected porosity enables for relatively significant cargo loading, such as proteins and live cells [177]. Combining porous architecture with electroactive materials can be beneficial in controlling the molecule release through electrical stimulation [178], and even be used simultaneously for electrical therapy [179].

1.5.4 Hydrogels

With respect to materials that can be processed in a variety of morphologies with tailored properties and responses, particular mention must be devoted to hydrogels. Throughout the past two decades, hydrogel-based matrices have been among the most popular scaffolds for TE. While not confined to a specific architecture or morphology, hydrogels encompass natural, synthetic, or composite polymeric cross-linked networks. Their resemblance to the native ECM in terms of high water content, flexibility, and elasticity contributes to their increasing use. Hydrogels offer easy customization regarding mechanical characteristics, enabling processing into diverse forms such as films, fibers, and 3D structures. Additionally, they can be loaded with biochemical factors, molecules, and materials to fine tune their performance, can fill any space, and are designed to be implanted through injection, avoiding invasive surgical procedures [133, 180]. These great advantages make hydrogels highly studied for the regeneration of all types of tissues.

Hydrogels can be synthesized using physical or chemical cross-linking, leading to a 3D network structure with unique properties suitable for a wide range of applications [181]. Physical cross-linking methods (e.g., temperature-induced, ionic, molecular entanglement) involve the use of non-covalent interactions between the polymer chains, such as hydrogen bonding, crystallization, protein, hydrophobic or ionic interactions, among others. Hydrogels cross-linked physically are reversible and responsive to external stimuli, such as pH, temperature, and ionic concentrations. On the other hand, chemical cross-linking involves the formation of covalent bonds between polymer chains, resulting in permanent and stable networks with lower responsiveness to external stimuli but higher mechanical strength. Chemical cross-linking methods include the use of chemical reactions, cross-linking agents, and radiation. Both physical and chemical methods possess their advantages and limitations, and their selection is dependent on the specific requirements of the hydrogel application [182].

One or more elements within a hydrogel's polymeric network structure can retain electroactive properties, resulting in a stimuli-responsive hydrogel, more specifically an electroresponsive hydrogel [183]. Electroresponsive hydrogels are frequently used for drug delivery purposes [184–186], but they can also be found in regeneration applications [187, 188].

1.6 Final Remarks

Electroactive biomaterials have emerged as a highly potential and needed approach to complement traditional methods for tissue repair and regeneration Given the presence of electrical and mechanoelectrical stimuli in various tissues of the human body, the use of smart materials, and in particular electrically conductive and piezoelectric ones, has shown to be a promising approach for tissue regeneration. This kind of biomaterials not only provide cellular support but also actively interact with the surrounding cells, leading to a more biomimetic recreation of the natural tissue microenvironment.

In this context, the primary focus should be on tailoring the biomaterial's morphology to match the specific tissue type being treated and the specific electrical signals to be statically and/or dynamically delivered. This approach considers the unique characteristics of the targeted tissue. In this way, developing an active biomaterial with an appropriate morphology capable of delivering physical stimuli to the target tissue, presents a needed and promising option for tissue repair treatments of specific tissues.

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