

Review

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Review

Recent Advances on Electrospun Fibers for Biological Applications

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Abstract: Electrospinning is a simple versatile method to generate nanofibres. Remarkable progress has been made in the development of electrospinning process. The production of nanofibers is affected by many parameters, which influence the final material properties. Electrospun fibers have a wide range of applications such as energy storage and biomedical scaffolds. Polyacrylonitrile (PAN) has been widely used in the production of carbon nanofibers. PAN has received increasing interest in recent years, due to its excellent characteristics, such as spinnability, biocompatibility, and commercial viability, opening new applications in the biotechnological field. This paper provides an overview of the electrospinning process, its main parameters, and operation modes that affect nanofibers fabrication. A review of their biological applications is proposed. A focus is made on PAN fibers formation, functionalization, and application as scaffolds to allow cell growth. Overall, nanofibre scaffolds, made of stabilized PAN, appear as a powerful tool in medical applications needing controlled cell culture.

Keywords: electrospinning; single needle; multi-needle; polyacrylonitrile; nanofibers; parameters; scaffold; biological application; tissues engineering; cell culture

1. Introduction

Fibers, in the form of continuous and elongated filaments, have been used by mankind since 2700 BC with the breeding of silkworms for textile production [1]. In Australasia, silk from spiders was used for fishing, while in ancient Greece, spider web was used as a wound dressing to stop bleeding. World War II saw an increase in demand for these fibers, leading to the production of man-made fibers derived from petroleum with sometimes better chemical or physical properties than silk, depending on the polymer [2,3] Nylon fibers, first introduced by DuPont, immediately caught the public's attention. In 2018, more than 50 million tons of synthetic fibers were produced, with a predominance of polyester filament. Therefore, many different types of synthetic polymers originating from crude oil, such as polystyrene and polyacrylonitrile (PAN), and natural biopolymers such as chitosan and polylactic acid, have been developed one after the other for various industries. Compared to synthetic fibers, natural fibers have many advantages thanks to their abundance, availability, and low cost, explaining why the global demand for synthetic fibers has been largely reduced, with synthetic fibers having been replaced by natural biofibers [4]. Nowadays, within the domain of fiber technology, nanofiber mats have a broad range of applications, especially in the areas of engineering and science. These include optical and chemical sensors, nano catalysis, and energy storage [5–7], as well as applications in defense, aerospace, transportation, and protective clothing industries [6]. They are also used in air and water filters or drug delivery for medical and biotechnological purposes [8].

To produce nanofibers, different methods have been developed such as wet, dry, melt, and gel spinning. During these processes, polymer jets are formed under external mechanical drawing/shearing forces when passing through spinnerets, and fibers are formed due to the solidification of the jets because of drying or precipitation. However, jets are only stretched to a

limited extent, corresponding to the formation of fibers with diameters ranging from 10 to 100 micrometers [9]. However, even with further mechanical drawing after solidification, the resulting fibers still cannot reach the nanoscale. In 1902, a novel technique similar to electrospray was created allowing fibers to be created at the nanoscale; this technique is known as electrospinning [10]. Both techniques rely on high voltage to eject liquid jets, the major difference being the viscosity and viscoelasticity of the liquid involved, and thus the behavior of the jet [11]. Electrospinning is a simple and low-cost method to prepare fibers at the nanoscale with an electric field. This method produces non-woven nanofibers mats, where fiber diameter ranges from ten to a hundred nanometers. Extraordinary properties such as small diameters or large specific surface areas can be obtained thanks to this technique. Also, a wide selection of polymers can be electrospun from diverse solvents, including aqueous solutions, making the spinning process itself environmentally friendly. Most toxic or corrosive solvents are used for waterproof polymers while only some can also be electrospun from a low toxic solvent such as dimethyl sulfoxide (DMSO) [12–14]. The classic electrospinning process uses one needle to draw the polymeric solution out and form fibers. However, this method is very time-consuming, limiting the potential scale-up application of the electrospun nanofibers. Many multiple jet [15] and multi-needle [16] electrospinning methods have been investigated to overcome the productivity problem, increasing the flow rate of the solution. However, the generation of multiple jets brings other problems such as jet repulsion and lower process controllability [17]. Polyacrylonitrile (PAN) is a synthetic polymer widely used in electrospinning due to its high tensile strength, thermal stability, carbon yield, and chemical resistivity. Electrospun PAN nano-fibrous membranes have received particular interest due to their appealing properties including small fiber diameters, as well as capabilities to control pore sizes among nanofibers and to incorporate molecules, cells, or proteins [14,18,19]. PAN is the primary precursor to prepare high-performance carbon fibers: almost 90% of the carbon nanofibers in the world are produced from PAN-based precursors [20]. Three main processes are involved in carbon fiber preparation: spinning of PAN precursor, thermal-oxidative stabilization (ToS), and carbonization [21]. Stabilization is a complex, exothermic process and the most important step that influences the properties of the final fibers. During this step, the linear polymer transforms itself into a cyclic structured polymer also called a ladder-like structure. Thermal stabilization proceeds mainly at a specific temperature ranging from 200 to 300°C for 1 – 2 hours in air [22]. Oxygen plays an important role in the physical and chemical structural changes during ToS. It is recognized that oxygen not only affects the oxidative and the cross-linking reaction but also makes the cyclization and dehydrogenation reaction (that occur simultaneously during the thermal stabilization process) easier. In addition, the exact reaction mechanism varies depending on the gas atmosphere, heating rate, copolymer used, etc [23,24]. Thus, having a better understanding of the structural evolution of PAN fibers during stabilization is a major issue to predict the final structure of the polymer and therefore to be able to functionalize it. In this study, a bibliographic review has been performed to better understand the electrospinning process including the principles, methods, and materials. In the first instance, the principles and typical apparatus of electrospinning are discussed. Next, methods and materials are described, and how the compositions, structures, and properties of polymer solutions can be engineered to obtain different materials is discussed. After that, a particular focus is made on 3D electrospun scaffolds produced for biological studies by first presenting their importance and the choice of materials, with as special focus on PAN fibers. Then, different processes that can be made to obtain relevant scaffolds will be outlined. Finally, different concrete examples will be highlighted. In the light of their exceptional properties and the many examples reported here, scaffolds of stabilized PAN nanofibers appear as an indispensable tool for the future development of cell culture for biomedical applications.

2. Electrospinning Process

2.1. Principle

The simplest form of electrospinning is single-needle configuration, which has been investigated and reviewed by many researchers [6,9,18,25–27]. Simulation of the electric field as well as modeling

of the electrospinning process were also carried out in the effort to provide a better understanding of this approach [28]. Only a few components are required: a syringe containing a polymeric solution, a metallic needle, a high voltage power supply, a metallic collector (with variable morphology). The power supply can be either direct current (DC) or more rarely alternating current (AC) [25]. As illustrated in Figure 1, the basic setup for electrospinning is rather simple, making it accessible to as many researchers as possible.

Free charges carried by the liquid interact with the applied electric field. The tensile force-inducing fiber jetting is due to the potential difference between the charged liquid in the spinneret and the grounded collector. Usually, electrospinning unfolds four steps (Figure 3): (i) the liquid droplet is charged, and the Taylor cone is formed; (ii) the charged jet is extended along a straight line; (iii) the jet is thinned in presence of an electric field as well as electrical bending instability appears and increases; (iv) finally, the jet solid fibers is collected on a grounded collector [17,25].

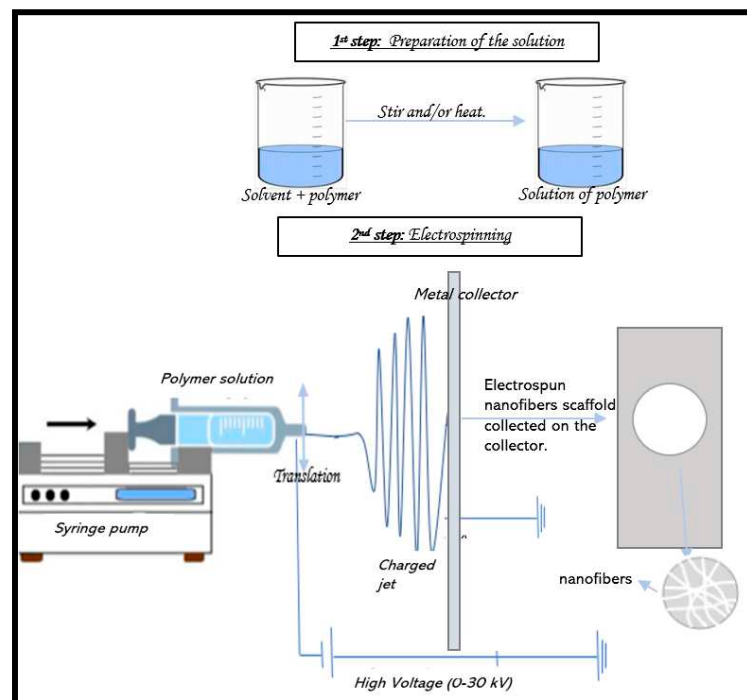


Figure 1. 1 Schematic diagram of processing steps for the fabrication of nanofibers. The polymeric solution is composed of solvent, polymer, and in some cases, additives such as nanoparticles or carbon nanotubes.

2.2. Formation of Taylor Cone upon charging a liquid droplet

A syringe with a needle is commonly used as the spinneret to feed the solution, at a controlled rate using a syringe pump. When there is a potential difference (ranging from 10 to 30 kV) between the spinneret and the collector, negative and positive charges will be separated within the liquid; the charges with the same polarity than the spinneret will migrate toward the droplet's surface, leading to an excess of charges. By increasing the voltage, more charges will accumulate at the liquid surface. This increase of the density of surface charges tends to deform the shape of the droplet, leading to an increase of surface area to attenuate the electric repulsion [29] as illustrated in Figure 2.

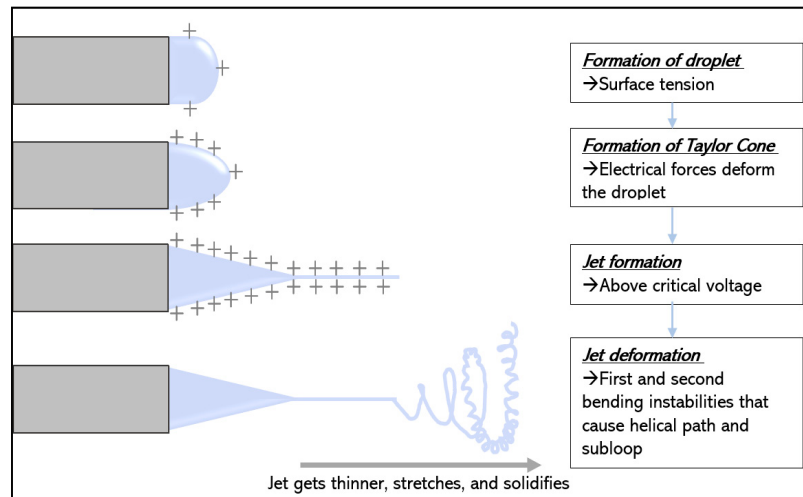


Figure 2. The different steps of the electrospinning process. A droplet is created due to the syringe pump. The surface area of the droplet in-creases to decrease the electric repulsion. A jet is ejected from the nozzle, and binding instabilities lead to the stretching and thinning of the jet. Finally, the solvent is evaporated, and the fibers are collected on a metallic collector. Inspired from reference [17].

2.3. Stretching and thinning of the charged jet

At the same time, the reciprocal repulsion of charges produces a force that opposes the surface tension, and ultimately the polymer solution flows in the direction of the electric field. A further increase in the electric field causes the spherical droplet to deform and assume a conical shape, which is called the Taylor cone. At the same stage, ultrafine fibers emerge from the conical polymer droplet at high speed, accelerated by the electric field. The jet will be extended in the direction of the electric field and then it is collected on the metallic collector kept at an optimized distance [9].

The jet formed initially travels in a straight path due to surface tension and viscoelastic forces which tend to prevent the jet from moving in the other directions. The acceleration of the jet decreases while the diameter of the jet also decreases as the jet is continuously stretched. Solvent evaporation and jet diameter reduction lead to an increase in surface charges density of the fiber which results in increasing repulsive forces in the jet. When the acceleration of the jet is too low, bending instabilities appear [9] and are described as long waves perturbation to the jet, driven by a lateral electrostatic force in radial direction relative to the jet, resulting from the electrostatic repulsion among surface charges [29,30].

The mechanism for the bending instability can be explained using Earnshaw's theorem illustrated in Figure 3. It states that a charge cannot maintain a stable equilibrium condition by relying only on the interaction between charges. If three adjacent points of equal charges in a straight jet are considered, the forces F and F' acting on the middle charge are of equal magnitude and in opposite directions following the equation:

$$F = k \frac{q_1 q_2}{R^2} = \frac{q_2 q_3}{R^2} = F' \quad (1)$$

where q_1 , q_2 and q_3 are point charges of equal magnitudes, R is the distance between charges, and k is Coulomb's constant ($k = 8.99 \times 10^9 \text{ Nm}^2/\text{C}^2$).

In the straight segment, the flow direction (which is the trajectory of a segment of the jet) is parallel to the axis of the jet. When a small perturbation causes a movement of the middle charge out of the straight line, the bending perturbation begins. An angle, θ , is created, which results in a lateral force F_R leading to the initiation of a jet instability given by:

$$F_R = 2F \sin \theta \quad (2)$$

This perturbation grows rapidly under the influence of the charge carried with the jet. It leads to elongation, reducing the diameter of the jet. After the first bending instability, the jet is prone to be subjected to subsequent higher order bending instabilities, leading to the formation of subloops. These loopings significantly contribute to the thinning of the jet for the formation of the nanofibers.

A succession of three or smaller diameter bending instabilities is often observed before the jet solidifies, as shown in Figure 4 [17,25].

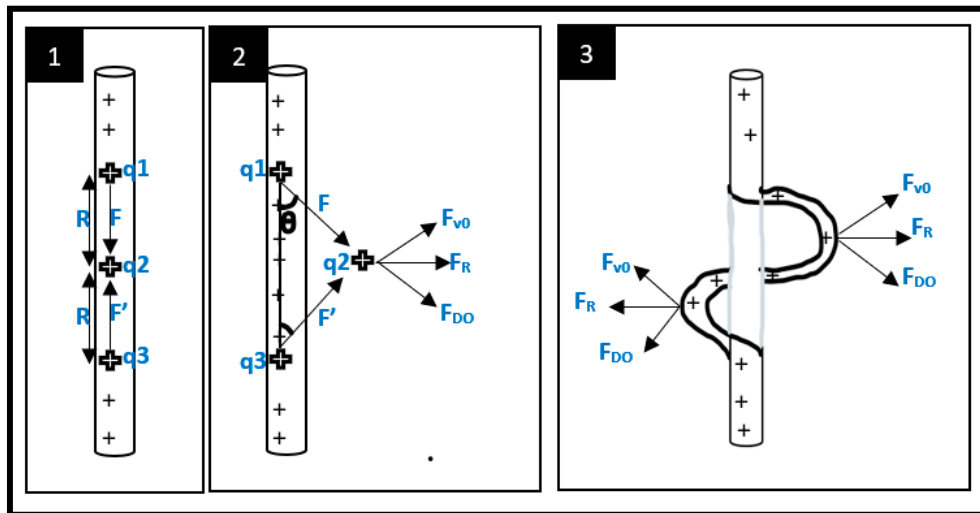


Figure 3. Representation of binding instabilities also called whipping instability, illustrating Earnshaw's theorem. First, the jet propagates in a straight line, highly accelerated due to the electric field. As the acceleration and the fiber diameter decrease, the surface charges density increases leading to an increase in repulsive force F and F' . A perturbation (represented by dotted lines) begins to grow in response to the repulsive electric forces. The charges carried by the perturbed segment are forced (F_{DO}) downward and outward by the charges above and below the perturbed region, while this perturbed segment is forced (F_{v0}) upward and outward by the charges below the perturbation. The resultant of these F_R forces is perpendicular to the straight jet and grows exponentially with time. At the same time, the repulsion of adjacent charges moves with the jet, propagating and amplifying the phenomenon. The elongation of the fibers increases more rapidly in the curved segment, creating nanofibers. Inspired from reference [29].

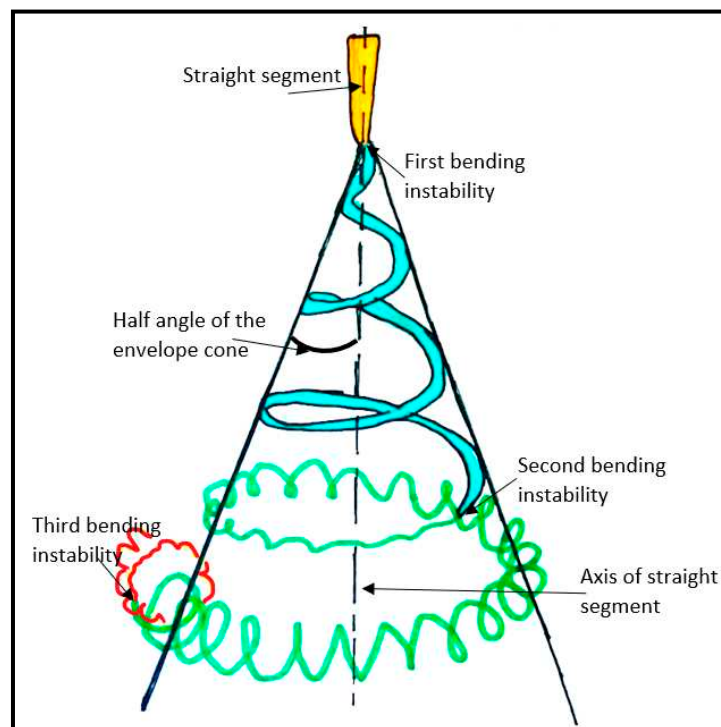


Figure 4. Typical path of electrospinning jet. It starts with a straight segment (in yellow), follow by a coil of decreasing diameter (first binding coil in blue). After several turns were formed, a new

electrical instability forms a smaller coil on a turn of the larger coil (second bending instabilities in green). The turns of the smaller coil transform into an even smaller coil (third bending instabilities in red). Usually, the solidification of the thin jet helps to stop the elongation. Inspired from reference [29].

2.4. Deposition of solid fibers

While the jet stretches and becomes thinner as it travels further under the influence of electrostatic forces, it undergoes solidification due to solvent evaporation. After solidification, some can find charges still trapped on the surface of the dry fibers, but all instabilities will stop [25].

In general, a grounded collector is used to collect fibers. The morphologies of fibers are mainly determined by the whipping in-stability. After deposition, most charges will be dissipated through the grounded collector. However, due to the low conductivity of most materials for fibers, a residual number of charges can still be found on the surface of the collected fibers [31]. Different collectors can be employed to obtain various alignments of fibers. The simplest and most common collector is a flat plate of alumina, leading to a non-woven fibrous mat. Different geometries can be obtained using different shapes such as rotating drums or parallel electrodes to obtain aligned fibers. In this case, optimization of rotating speed and voltage applied needs to be done [17].

2.5. Control of the electrospinning process

The formation of electrospun nanofibers is determined by the processing parameters, including the voltage applied, the flowrate of the polymeric solution, the distance between the syringe tip and the collector, and the diameter of the needle. Other parameters such as environmental parameters as well as solution parameters also play a significant role in the fabrication of nanofibers.

2.5.1. Effects of the voltage:

We have seen above that a high voltage applied to a solution in a metallic needle will induce the deformation of a spherical droplet into a Taylor cone and the formation of nanofibers when reaching a critical voltage. The latter depends on the polymer used and it ranges from 10 to 30kV. It is accepted that the higher the voltage, the smaller the diameter of the fibers, due to the correlation between charges repulsion within the polymer jet and the stretching of the polymer [32]. Nevertheless, Son et al. report-ed that an increase in the voltage applied beyond the critical voltage will result in the formation of beads or beaded-nanofibers [33].

2.5.2. Effects on the flowrate:

Higher flow rates will undoubtedly increase the production rate of the electrospinning process but can have negative effects on the morphology of the fibers if not properly controlled. Uniform nanofibers can be prepared via an optimal flow rate for a mixture. Flowrates generally range from 0.1 to 4.5 mL/hour depending on the polymer used [17]. When flow rate is above this critical value, formation of beads appears, which is due to incomplete drying of the nanofiber jet during the flight between the needle tip and the metallic collector and a decrease of the surface charge density [26].

2.5.3. Effect on the distance between metallic collector and needle:

The distance between the nozzle tip and the collector is an important parameter to produce excellent quality nanofibers. The nanofibers morphology can be easily affected by the distance (which usually ranges from 5 cm to 20 cm [34]) because it depends on the evaporation rate, and the binding instabilities of the polymeric solution. A minimum distance is required to allow sufficient time to complete solvent evaporation, to prevent defects like beaded or flattened fibers. A distance collector-needle too small results in a small circular area with a deep color called "wet spot" which can be found in a center of the mats [35]. Like the applied voltage and the flow rate, this minimum distance also varies with the polymer system. However, Zhang et al. observed no significant changes in

polyvinyl alcohol (PVA) fiber morphology while varying the needle-collector distance from 8 to 15 cm [36].

2.5.4. Effects of needle diameter:

Stainless steel needles are the most used nozzles in electrospinning, with a typical inner diameter ranging from 0.2 to 1.5 mm. Generally, a large needle diameter will lead to a large fiber diameter. The increase of fiber diameter results in the fact that with a larger tip, a larger droplet is formed and thus a longer length of the straight jet, which in turn reduces the whipping jet motion. Clogging of the tip can also occur when the needle diameter is too low, and the solution is too viscous; or when the needle diameter is too large, and the solution tends to solidify with increased exposure to air [26].

2.5.5. Effects of polymer concentration in solution:

The electrospinning process relies on the phenomenon of the uniaxial stretching of the charged jet. The jet depends on the arrangement of the polymer particles in the solution, which is a direct consequence of the solution concentration. For example, when the concentration of polymer is too low, the entanglement of polymeric chains is limited, breaking fibers into fragments and leading to beaded fibers. Increasing the concentration will increase the viscosity, which increases the chains' entanglement among the polymer chains. This overcomes the surface tension and results in the formation of defect-free fibers [25]. The molecular weight of the polymer also plays a key role. Jacobs et al reported for poly (ethylene oxide) (PEO) of two different molecular weights (300 000 & 900 000 g/mol), that smooth fibers are formed at 6wt% with a higher molecular weight, while a higher concentration is required for the solution with lower molecular weight [37].

2.5.6. Effect of solution conductivity:

Solution conductivity affects both Taylor cone formation and fiber diameter. For a low conductivity solution, the surface of the droplet has no charges to form Taylor cone, and then the electrospinning process cannot take place. Increasing the conductivity with the addition of surfactants or salt will increase the charges on the surface of the droplet to form Taylor cone and decrease fiber diameter. Several types of salt have been used, with varying amounts (generally up to 2%). Above 2%, the jet becomes un-stable, which inhibits the electrospinning process [26]. Son et al. studied the effects of Poly (allylamine hydrochloride salts) (PAH) and poly (acrylic acid sodium salt) (PAA) on PEO nanofibers. By adding only 0.1% of each salt (PAH and PAA) the diameter of PEO fibers was reduced by 55%, from 360 nm to 200 nm [33]. This shows that adding surfactant and salt increases the conductivity of the solution, and the increased electric field force improves the binding instabilities of the jet, which aids the stretching and thinning of the nanofibers.

2.5.7. Solvent effects:

The selection of the solvent is one key parameter for the formation of homogeneous, smooth, and beadless electrospun nanofibers. First, the polymer needs to be completely soluble in this solvent, a homogeneous solution is essential for the electrospinning process. However, a solvent with a high solubility parameter does not necessarily produce a solution suitable for the electrospinning [33]. Secondly, the solvent should have a moderate boiling point, which determines the evaporation rate and thus the solidification rate of the jet. An extremely high volatility is not desirable because the jet may solidify immediately at the needle tip, blocking the spinneret and stopping the electrospinning process. If the volatility is too low, the fibers will still be wet when they are deposited in the collector, which can lead to the formation of beaded nanofibers [26].

The dielectric constant also plays a significant role because it controls the magnitude of electrostatic repulsion among the surface charges residing in the jet. Increasing the dielectric constant implies increasing the applied voltage, to achieve a stable jet. Therefore, water is not a favorable solvent for electrospinning, due to its high dielectric constant and thus the attenuation of the electrostatic repulsion. The commonly used solvents in electrospinning are alcohol, dichloromethane,

chloroform, dimethylformamide (DMF), tetrahydrofuran (THF), acetone, dimethyl sulfoxide (DMSO), hexafluoro isopropanol (HFIP), and trifluoroethanol. In some cases, it can be interesting to mix two solvents, to achieve an optimal formulation for electrospinning [25]. Nevertheless, the use of two solvents will have an impact on the fiber's porosity: the different evaporation rates of the solvents will lead to phase separation and hence will result in the fabrication of a porous material [26].

2.5.8. Effect of the targeted drum size and its speed of rotation:

The morphology of the fibers is influenced by the nature of the collector. The choice of collector type will be guided by the area of fibres wanted- a drum collector will give a higher area of aligned fibres than a disc collector for example. To obtain aligned fibres, a rotating collector, an array of electrodes or a pair of magnets is required. When the collector is an array of electrodes, the alignment will be tuned by acting on the electric field, whereas alignment will be controlled by the magnetic field when the collector is a pair of magnets. By modifying rotation speed of a rotator collector, it is the mechanical stretching forces that will influence alignment of fibres [26,38]. Usually, when the rotation speed is under 1 000 rpm, the mesh is randomly organized. Above 500 rpm, the higher the speed is, the better the alignment is because polymer molecular chains will be more aligned according to fibres axis and so the crystal orientation of fibres will be improved [39,40]. For example, the impact of rotation speed was studied for POM (polyoxymethylene) nanofibres, POM being a highly crystalline polymer. It has been shown that the morphology of the nanofibres changes from a folded chain crystal to an extended chain crystal when the rotation speed of the drum collector increases. [41] In another study, four speeds were tested (500, 1000, 1500 and 2150 rpm) to obtain electrospun nanofibres of PVA. For 500 and 1000 rpm, Andreas et al. obtained random orientation while for 1500 and 2150 rpm, the orientation was more organized. [42]

2.6. Multineedle for large scale production

One major challenge electrospinning is facing nowadays is the upscaling to answer the industry demand. In most published work about electrospinning, authors have used experimental laboratory setups using a single syringe as described above with an inner diameter of 0.2 to 1.5 mm. Depending on the polymeric solution, the flow rate is typically limited to 5 milliliters per hour. Consequently, nanofibers are obtained at a very low yield, which limits the industrial application of electrospinning.

The most important parameter for high efficiency in the electrospinning process is the flow rate. However, the flow rate is largely determined by the strength of the electric field. Increasing the flowrate means increasing the voltage, which will lead to the formation of the droplet and no longer of the fibers (see Control of electrospinning process, Effect of voltage), and an increase of the nanofiber's diameter (see Control of the electrospinning process, Effects of flowrate). Therefore, it is not feasible to substantially increase the throughput of the single-needle electrospinning process where a compromise must be made between the applied voltage and the flow rate.

Some tried to use needleless electrospinning to increase production. However, some parameters, such as solvent evaporation, are easily variable, which makes it difficult to obtain accurate and reproducible fabrication at industry scale production.

To overcome the imperfections of single-needle and needleless electrospinning, multi-needle electrospinning can be an important choice of nanofiber production. The combination of several individual needles as the spinneret of the electrospinning setup is the most direct method to increase productivity. This method is also useful to produce composite fibers of two or more polymers where there is no common solvent.

2.6.1. Multi-needle electrospinning process:

- Principle and properties

The working mechanism of a multi-needle electrospinning process is very close to the conventional single-needle electrospinning process. For a single jet emitted from a single needle, the

initial straight path of the jet is driven by forces in the direction of the electric field. For the multi-nozzle setup, multiple jets can be simultaneously ejected from the needles, and all of them then undergo a bending instability. The jets are influenced by Coulombic forces F_c , exerted by jet 's neighbors, in addition to the electric field force F_e . The deviation of the jet is the result of these two forces, as shown in Figure 5a [17,43].

In the multi-needle electrospinning setup, the inter-nozzle distance is usually set at 10–50 mm, which causes complex effects in the electrospinning process. When the distances between needles are relatively small, the mutual interferences of the electric field of the tips will be larger, which may be the cause of uneven electric field, which directly affects fibers quality [27].

Many factors affect the electric field, including needles configuration, needles number, and needles spacing. The spacing is mainly affected by the diameter of the needles and the properties of the polymeric solution. As shown in Figure 5b increasing the inter-needle distance decreases the repulsion among jets, while pushing the jets closer resulting in concentrating the fibers created. The number of needles arranged over a certain area is dependent on both the spacing and the layout, and it ultimately determines the throughput of fiber production. The layout of the array controls the distribution of the electric field, to obtain the most uniform electric field among the jet as possible [25]. The configuration of multiple needles can be divided into two types: linear arrays, by organizing needles in a straight line [28], and two-dimensional arrays by placing needles in a special layout, including square [38], circular elliptic [39], hexagonal [43], and triangular patterns.

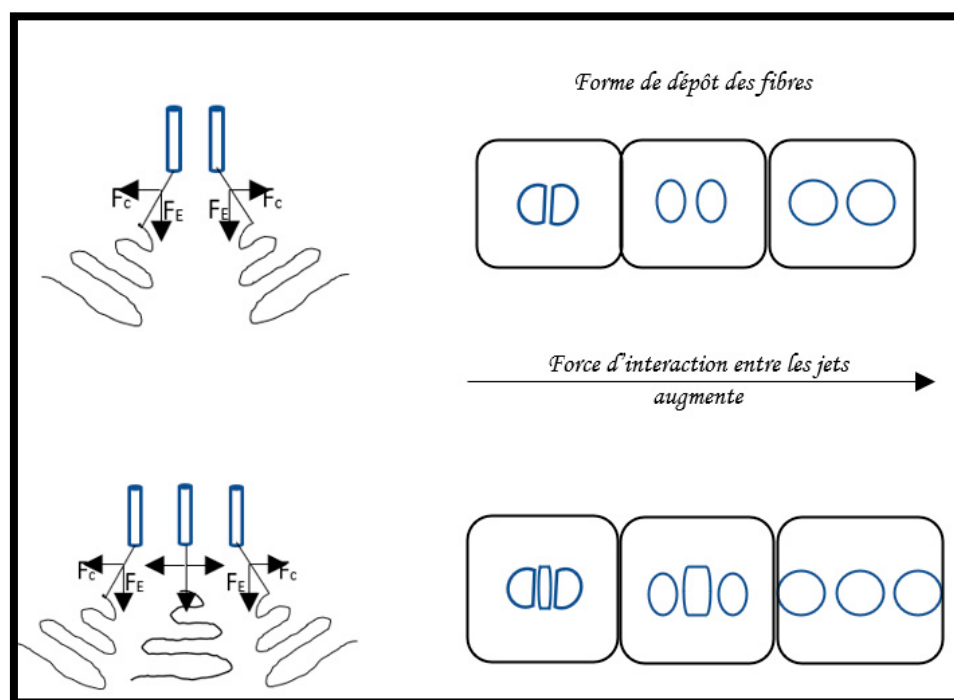


Figure 5. Force analysis in multiple-nozzle electrospinning with typical fiber deposition shapes. In the three nozzles configuration, the path of the central jet develops in the same way as that in the single-jet electrospinning, due to the symmetrical arrangement of the side jets, while the paths of the outer jets are deviated by Coulomb force. Besides the electric field force F_e , the Coulomb forces F_c are exerted on each jet by their neighbors. The higher the voltage, the further away the patterns will be. Inspired from reference [17].

Several examples of multi-needles in a linear array as the simplest arrangement have been reported. For instance, linear multi-needle electrospinning set up with four needles has been designed to produce nanofibers webs.

Zhu et al. showed that the charge density distribution of the needles and the morphology of the jet vary, depending on the number and the placement of nozzles used in space. For a single needle, the charge distribution of a single needle is uniform and mainly distributed at the end of the tip.

Contrarily, in the double-needle layout, when a needle is added next to another one, they share the same voltage, and the same charges will repel each other. Then, uneven charge density distribution appears, the charges density outside the tips are higher than the charges on the adjacent part of the two-needle tip. In the three-needle electrospinning process, the electric field of the middle tip is affected by the electric field on both sides, and this phenomenon results in uniform charge distribution in the middle tip, and non-uniform charge distribution in the two other needles. Experimental and simulated results showed that the behavior of border jets along the linear array was different from that of the central jet, such as bending direction and envelope cone. However, every jet in both linear configurations was subject to the characteristic bending instability similar to that in single-jet electrospinning [38].

In addition, the outer jets showed a longer length for the initial straight region and a larger size for the envelope cone than the central jet, due to the progressive weakening of the electrical field from the edge to the center. It is observed that increasing the voltage also increases the straight lengths and the envelope cones of the central jet and the outer jet, as well as the deviation angle of the outer jet. However, if the voltage is too low, the electric force is not strong enough and the jet cannot eject from the central needle [43].

Most investigations on multi-needle electrospinning have been focused on processes with two-dimensional needle arrays. For example, multi-needle spinning with elliptic and circular arrangements were designed to improve the process stability and the production efficiency [39]. Yang et al. used a hexagonal array composed of seven needles, with one needle located in the center. They found that the needle at the center behave as in normal electrospinning process, while the jet from the peripheral nozzle is heading outwards, due to repulsive electric forces [40]. A complex design has been created by Theron et al. with a 3x3 needle matrix and they succeeded in reaching $22.5 \text{ L.cm}^{-1} \text{ min}^{-1}$ due to their nine-needle electrospinning process. This nine-needle square configuration is described as stable and uniform fibers have been obtained [41].

- Control of fiber formation in multi-needle electrospinning setup.

Several disadvantages of the multi-nozzle system are repulsion by adjacent jets and the non-uniform electrical field at each nozzle tip of the spinneret. Repulsion at the jets leads to difficulty in the collection, while a non-uniform electric field gives processing problems. Some research on electric fields has already been done like the Chan et al. work, which considers the effect of linear-array nozzle configurations (the angle between the nozzles) on the electric field distribution observed, to solve the electric field problem. Three angles were tested: 180° , 100° , and 90° . Simulations and experimentations show that decreasing the angle between the nozzles increases the electric field strength, and it is well known that increasing the electric field will lead to a decrease in fiber diameters. Therefore, fibers electrospun with an angle of 90° will have a lower diameter (about 25% reduction) than fibers electrospun with an angle of 180° [28].

In the case of many needles, a decrease in needle spacing increases the deposition rate over a small area. However, it leads to an increase in electric repulsion between jets. That's why controlling the distribution and the electromagnetic field in the working space is essential. Both can be manipulated using auxiliary electrodes. In single-needle electrospinning, auxiliary electrodes are used to produce multi-jet [15]. Auxiliary electrodes (also called secondary electrodes) with the same polarity as the needle tend to concentrate the electric field, reducing or neutralizing the repulsion among the jets. Different auxiliary electrodes shapes (Figure 6) have been tested to try to control the jets. Hexagonal array (Figure 6a) within a cylindrical auxiliary electrode [17] tends to reduce electric field interferences and stabilize the jet motion. Thicker fibers were obtained due to the secondary electrode, which interferes with the jet and thus the bending instabilities, leading to a decrease of fibers stretching. Contrary to Figure 6b, an auxiliary ring electrode [42] provides more uniform electric field near the nozzle tips and greater mean electric field strength in the space between the nozzles and collector. It leads to tinner and more uniform fibers. In Figure 6c, a flat plate [17] was used as a secondary electrode. By adjusting the position of the flat plate, smaller fibers diameters and more concentrated fibers were observed when the electrode was aligned at the edge of the needle, resembling a flat spinneret with holes.

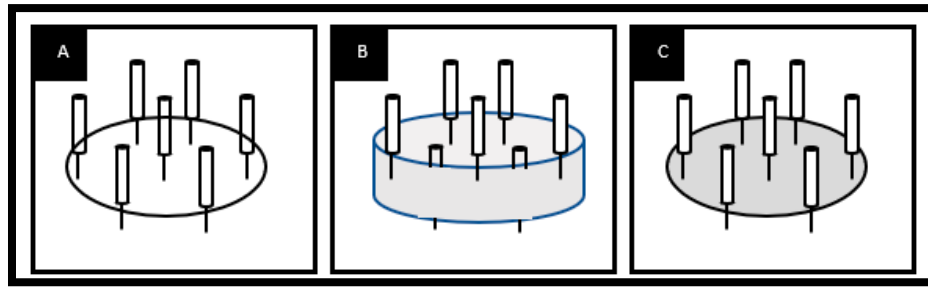


Figure 6. Different types of auxiliary electrodes in multiple-nozzle electrospinning: (a) Ring electrode, (b) cylindrical electrode, and (c) plate electrode. Inspired from reference [17].

2.6.2. Other approaches

Some other strategies are explored [44]. First, nozzles technologies. When applied voltage reaches a critical value, simultaneous multiple jets are formed. For this method, two spinnerets can be used. Either the rotating spinneret, which induces a mechanical rotation of the solution, or the stationary spinneret, which needs an auxiliary force such as a magnetic field. Then, alternating current electrospinning has been shown to increase the production of nanofibers six-fold. Another method is ultrasound-enhanced electrospinning. It was used to produce PAN nanofibers and the production rate was improved.

2.6.3. New directions for future development

Despite many reports on the successful demonstration of electrospinning as a reproducible and versatile method, the main issue is a large amount of solvent used, resulting in both economic and environmental concerns. The solvent represents about 80 to 90% of the solution involved in traditional electrospinning. During the process, the solvent is lost while the fibers are stretched, and it is generally not collected or recycled. Finally, there is a critical demand to develop electrospinning process based on “green” solvents or even solvent-free systems.

In general, single-needle electrospinning is only suitable for laboratory use as it suffers from low productivity. To solve this issue, innovative modifications to the conventional electrospinning setup have been made, including a multi-needle electrospinning process. But for this last one, future efforts should be concentrated on solving the problems related to undesirable interactions, such as the generation of inhomogeneous fibers or poor fibers distribution in the collected mat.

3. Electrospinning of Scaffold for biological studies

Electrospun membranes are used in various fields such as biology, nanosensor fabrication, protection, information storage, and transport, energy, textile, absorption of irradiation, decomposition of contaminants, food industry and electrospinning process filtration [45].

In biology, these scaffolds allow the production of tissues, the delivery of drugs, the production of dressings, immobilization of enzymes, and the obtention of 3D scaffold for cell culture [45].

3.1. Why electrospun 3D scaffold cultures?

3.1.1. 3D cultures versus 2D culture

When seeded in 2D culture, predictability and results are not representative of what happens in vivo [46]. In fact, cells have been presented as losing their differentiation, present neoplastic properties, and great differences in transcriptomic are observed. For instance, the expression of 90% of genes involved in cell adhesions, extracellular matrix (ECM) remodeling, and tissue development is excluded in melanoma cells seeded in 2D compared to in vivo 3D tumors [46]. In another study, 2D culture of GICs imposed an apico-basal polarity which is not relevant in concept of GBM and is abolished when cultured in 3D environment. When seeded on the 3D scaffold, galectin-3 and

integrin- β 1 were upregulated, which induced mesenchymal migration of GBM cells, similarly to what happen in vivo [47]. 3D culture, for its part, by mimicking better the physiological environments, allows differentiation of the cells with respect for the tissue of origins. The results relevance in 3D about cellular morphologies, differentiation and proliferation properties, gene expression, and sensitivity to treatment have been extensively demonstrated while maintaining heterogeneity of both cells and transports of nutrients and oxygen [46,47]. One major challenge for tissue engineering and production of 3D cell culture scaffold is to produce and monitor a microenvironment that mimics as effectively as possible the in vivo ECM with fine control of mechanical and chemical properties, allowing limitless imaging and complex analysis with the same convenience as in 2D. Every biological event, such as differentiation, migration, or proliferation, is impacted by the topography of the scaffold, its mechanical, physical, and chemical properties. as well as the availability and conformation of ECM protein deposition. The understanding of cells behavior is therefore essential to be investigated in the environment as close as possible of the one where each cell type is evolving in vivo [25]. Nevertheless, the current 3D environment available on the market presents numerous limitations.

3.1.2. 3D scaffolds

Among the last decade there is increasing number of artificial three-dimensional tissue surrogates that have been developed.

Hydrogels,[48] made of highly hydrated polymers, and are mimicking ECM native environment. They can be made up from natural polymers (such as collagen, chitosan, alginate, hyaluronic acid), or synthetic polymers (such as PEO, PVA, PAA or P(PF-co-EG), polylactic acid (PLA), polylactic-co-glycolide (PLGA), and polycaprolactone (PCL)). Despite their good properties, such as abundance and high similarity with ECM components for natural polymers or high tunability for synthetic polymer,[49] some limitations cannot be ignored. On firsthand, because of too small mesh area, reticulation of hydrogel on cells is necessary [50]. On second hand, natural polymers have a high rate of biodegradability and are variable which make it difficult to compare studies [51]. A polymer's molecular weight is an important parameter since it determines its activity and its effect on cell behavior and viability. Biodegradation of the polymer leads to a decrease in molecular weight which induce a complete change in cell microenvironment [52]. Poorly reticulated hydrogels have a degradation rate too high and do not allow long term cell culture, whereas highly reticulated hydrogels are too cytotoxic and with poor diffusion [51]. Consequently, physical properties of the environment are difficult to modulate without encountering associated limitations. As well homogenous distribution of nutrients, oxy-gen or drugs treatments is highly depending on the crosslinking as it influences the diffusion through the hydrogels, as well as the cell seeding method permitting to cells to be embedded inside the hydrogel [53]. Finally, omics analysis is hard to perform on cells seeded on hydrogel. Biodegradation induces the presence of wastes that may impact the results, treatments steps to harvest cells lead to modifications in genes expressions and yields of RNA extraction/purification are low [53].

Porous structure can be obtained by freeze-drying or leaching of biomaterials solution [48]. They are called sponge or foam scaffolds. Sponges are extensively used in the context of bone graft, permit growth et differentiation and present simple functionalization [54]. However, rigidity, porosity and degradation rate are not easily monitorable and tunable, visibility in microscopy without cryosection is poor, and in the context of cancer, is not designed to observe migration easily [54].

Ceramic scaffolds are used as biomimetic ECM environment for bone tissue specially as well. They are highly biocompatible since they are found in natural bone and they have osteoconduction and osseointegration properties [55]. However, they show lower mechanical strength and higher degradation rate than formation of new bone [54].

Fibrous scaffolds [48] can be obtained by electrospinning, molecular self-assembly or thermally induced phase separation. Not only does they enhance cell adhesion, growth, and migration but they also facilitate bioactive molecule diffusion within the scaffold are biocompatible, show good and tunable mechanical properties and surface chemistry and controllable biodegradability [51]. As well,

as composed of fibers, fibrous scaffold are offering morphological similarities to native ECM, composed by fibers too (collagen, laminin, fibronectin, hyaluronic acid, etc...) [56]. They are compatible with most of the microscopy technique, with live imaging and most of the histology and biochemistry protocols [47]. Studies realized on decellularized tissues are the closest from in vivo environment as they are 3D scaffolds based on the natural ECM, growth factors and tissues that are specific of the organ where the studied cells are coming from [57]. However, the availability is low for intact tissues, and it is hardly reproducible since it is particularly challenging to eliminate all cells residues without disturbing the overall organisation.

Organoids and spheroids are 3D self-organized and multi cellularized structures [58]. Nevertheless, they cannot be made from every cell type, and are not suitable for migrating studies in a physiological and pathological context alone [59]. Finally, they present low drugs penetration due to the absence of blood vessels. In this context, they would have to be combined with biomimetic environment such as nanofibers mat or microfluidic devices [59]. For instance, migration studies of neurospheres of GBM were performed on 3D nanofibers scaffold and results were more relevant than neurospheres seeded in 2D [47].

3.1.3. Production of 3D scaffolds

3D printing presents an important advantage since it is assisted by a computer that enables to control specifically every parameter such as the size and the localization of pores, the architecture, and mechanical properties. Bioprinting, for itself, is the printing of hydrogel ink with living cells in it. However, cells must bear stress and pressure during extrusion and usually the polymers used have low mechanical stiffness [48].

Electrospinning is the most widely chosen since it can provide scaffolds, composed of nano to microscale-diameter fibers, mimicking native ECM environment both at the micro and macro-scale [48].

3.2. Choice of materials

One key parameter to produce a biomaterial is the choice of material that must be biocompatible, and, to get an electrospun scaffold, the substance may be spinnable. Regarding these two criteria, one can choose either a polymer or a peptide, both being natural or synthetic.

3.2.1. Polymers

Organic polymers can be electrospun provided that the solvent allows a complete dissolution without degradation and that molecular weight is high enough [25].

Natural polymers can be chosen since they are biocompatible, recognized by cells faster, and have bio-functionality [60,61]. For example, chitosan has antibacterial and hemostatic properties that make it a proper candidate for wound healing. On the other hand, the fibrinogen scaffold presents better mechanical properties and a slow rate of degradation [62].

However, synthetic polymers are usually favored since they are less expensive, present better mechanical properties, and are more easily spinnable [60,61]. PCL, PLA, PGA, and PEO are the most common polymers used for electrospinning scaffolds for tissue engineering. PLA, for example, can degrade into lactic acid, mimics ECM structure and presents good mechanical properties. PEO is usually used to study soft-tissue environment [60].

Some polymers, such as polyethylene, are not dissoluble in solvents used for electrospinning [63]. It is melted and kept in this state by heating the needle from which the solution is expelled. When it happens, the polymer solidifies to form a fiber. One advantage is the absence of solvent residues at the end since this method does not use any solvent. However, this technique does not allow the obtention of nanofiber, and heating can degrade the polymer. Finally, the bioactive molecules cannot be incorporated into fibers with this technique.

Finally, composite nanofibers have the advantage to overcome some limitations by combining appropriate properties from the different components chosen [25]. For example, PVA/chitosan

scaffold developed by Agrawal et al. promoted cell proliferation and growth [64]. For sol-gel solution, hydrolyze, condensation, and gelation reactions are initiated in the jet in contact with the air. It allows having an inorganic phase in the fibers [65]. For example, Ag particles, which present antibacterial properties, were dispersed in PVA solution and could be therefore electrospun [66].

3.2.2. Specific case of PAN

Polyacrylonitrile (PAN) based fibers are the most suitable precursor to produce carbon fibers, compared to other precursors such as pitch or rayon, due to their high melting point and greater carbon yield (>50% of the original precursor mass). PAN fibers are also used on a large scale in the textile industry as acrylic fibers, and they are highly desirable for the high-performance composite, for automotive and aerospace technologies, due to their enhanced physical and mechanical characteristics [67]. PAN fibers can be used in the biological field, to promote cell growth, but so far, they are not widely widespread in this field. They are selected for their biocompatibility and the possibility to sterilize them thanks to the thermal treatment that stabilizes their structure [19]. They also show resistance to biodegradation, which not only does allow cell culture but also does complex omics analysis. The spatial design and mechanical properties of PAN nanofibers are tunable in an independent way versus other physicochemical parameters [19]. Finally, most waterproof polymers must be spun from toxic or corrosive solvents, while only some can also be electrospun from low-toxic solvents like dimethyl sulfoxide. Being spinnable from DMSO is one of the reasons why PAN is often used in electrospinning [13].

- Electrospinning of PAN

The electrospinning process of PAN has been widely studied in the few last decade's [12–14,18,68–73]. PAN's molecular weight most used is 150 000 g/mol. Only two solvents allow to make the electrospinning process: the N, N-dimethylformamide (DMF), and the dimethyl sulfoxide (DMSO); the first one is the most used but, in a few cases, DMSO is used as a low-toxic solvent [74]. In the DMF solvent, PAN percentage ranges from 6 to 14 wt%, depending on the fibers desired. The fiber PAN diameter increases with increasing polymer concentration; just as an increase in voltage, which ranges from 15 to 25 kV, increases the diameter of fibers. Although, the voltage influence was not as great as polymer concentration on the fiber's diameter. In the case of a too low concentration and voltage, Gu et al. showed that fibers spindle-like beads were obtained [75]. The collector to needle distance is between 15 and 25 cm. Generally, the typical diameter of PAN fiber ranges from 350 to 500 nm, depending on the parameters applied above.

- Thermal treatment of Polyacrylonitrile.

The thermal treatment of PAN, also called Thermal Oxidative Stabilization (ToS), is an essential and time-consuming step that modifies the original structure of PAN. This can be explained by chemical reactions that are involved in this process, which are cyclization, dehydrogenation, oxidation, and cross-linking reaction [76]. These reactions lead to the formation of a ladder-like structure, as shown in Figure 10. The order in which the reactions occur has long been discussed. It is now accepted that the cyclization and dehydrogenation reactions can take place at the same time, while the oxidation reaction can only take place after the obtention of the ladder structure [77]. ToS is a complicated stage since different chemical reactions take place at the same time. The step of stabilization converts C–N bonds to C=N which makes a non-melting stable ladder polymer structure from PAN fibers. The thermal stability of the fibers is assigned to cyclization of the nitrile groups [67]. During stabilization, PAN fibers color change, from white mats to yellow and brown to ultimately black fibers, depending on the time and the temperature of the stabilization. This darkening of color comes from the formation of the ladder structure [78]. In this process, temperature is a key parameter that would affect the heating treatment of PAN fibers. It usually ranges from 180 to 280 degrees [77,79] and most researchers performed stabilization at 250°C, as optimized temperature [76,80–82]. If the temperature is too high, the fibers can overheat and fuse or even burn. However, if the temperature is too low, the reactions are slow and incomplete, which does not lead to a ladder-like structure. The two most important reactions occurring during the ToS process are dehydrogenation

and cyclization, because of the structure modification of PAN. Both are important to form the final structure of stabilized nanofibers [67].

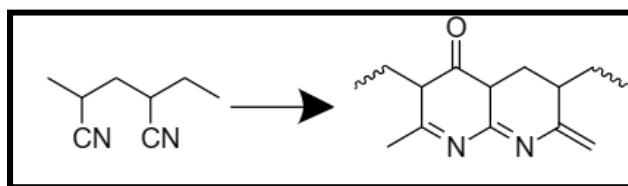


Figure 10. Reaction scheme of the PAN. The nitrile groups react together to form a ladder-like structure, highly stable due to dehydrogenation and oxidation reactions, which allow electron delocalization. Inspired from reference [67].

Cyclisation

The first reaction is the most important one because cyclization will determine the final PAN fibers' structure. Cyclization is the reaction of one nitrile group with the following one, to form a stable ladder structure. This reaction can be defined by the first-order kinetic equation and unlike dehydrogenation, cyclization reaction can be proceeded in either an inert gas (as nitrogen [83–85] or the presence of oxygen [86,87]. In other words, oxygen is not involved in the reaction mechanism of cyclization. This reaction is exothermic, and no weight loss is observed during this step [79,80]. This reaction is necessary to hold molecules in fiber together and increases the stiffness. Several sources can be involved in the occurrence of cyclization reaction. Impurities in the polymer, catalyst fragments and inhibitors can act as initiators of cyclization. It can also be started in chain end groups [77]. The initiation can start either with free radical or ionic mechanisms. The latter takes place when the precursor is a comonomer of PAN while free radical mechanism takes place when the precursor is an homopolymer. According to Kim et al. free radicals breaks the $C\equiv N$ bonds to $C=N$ bonds which leads to cyclization, as shown in Figure 11. Propagation is relatively rapid in free radical mechanism [83]. Nevertheless, a part of nitrile groups fails to convert into cyclic structures during ToS [76].

Fu et al. shown that cyclisation is more sensitive to isothermal temperature than isothermal time. For 250°C and 265°C, 70 % and 80 % of nitrile groups have reacted to form cycles. In fact, the linear PAN chains convert into cyclic structure by condensation between the adjacent two nitrile groups, which needs the conformation rotation of PAN. The higher temperature provides enough energy to overcome the barrier of conformation rotation of the linear structure [76]. To summarize, the higher the isothermal ToS temperature, the more cyclic structures form. Ruixue et al made eight stabilizations from 220°C to 280°C with a step of 10°C between each sample. They found that cyclisation reaction was complete at the temperature of 280°C (about 99% of theoretical cyclization), which generates a new intermediate cyclic structure, very similar to the (002) plane of the graphite structure [79].

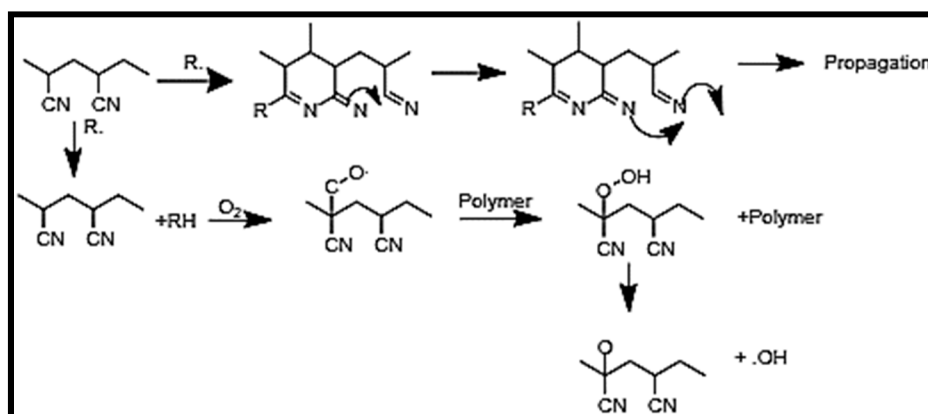


Figure 11. Free radical mechanism for the PAN homopolymer in air to form ladder-like PAN structure. Inspired from reference [83].

Dehydrogenation

Dehydrogenation is the elimination of hydrogen with oxygen in the form of water which leads to the formation of double bonds between carbon, that stabilize carbon chain by forming aromatic rings. The formation of the double bonds results in an increase of thermal stability of the polymer due to the decrease of chain scission (Figure 12) [67]. Fitzer et al showed with Differential Thermal Analysis (DTA) that dehydrogenation reaction in both air or nitrogen environment can happen before or after cyclization. Because of the use of oxygen in dehydrogenation reaction, concentration of oxygen plays an important role in the rate of this reaction. It has been proven that dehydrogenation happens almost at the same time as oxidation [77].

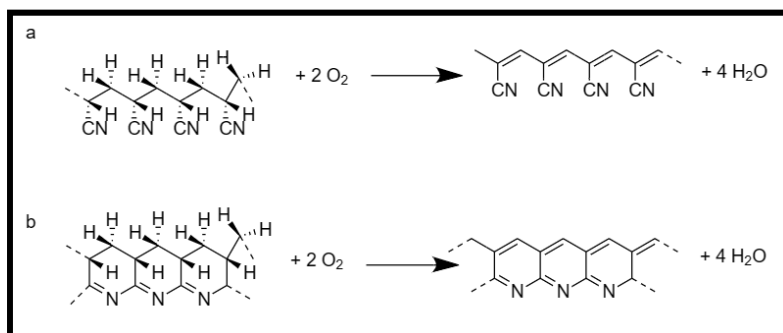


Figure 12. Reaction of dehydrogenation, (a) before cyclization reaction or (b) after cyclization reaction. Inspired from reference [67].

Oxidation

Oxidation reaction plays an important role in improving thermal stability of fibers by assisting in the evolution of aromatic rings and conjugated molecular structure. An oxidizing medium is necessary to allow the reaction to take place, which is typically air. Generally, the oxygen content in final stabilized fiber ranges from 8 wt% to 12 wt% [77]. Oxidation reaction, as well as dehydrogenation reaction, become dominant when the extent of cyclisation reaches 70% [76].

Figure 13 shows the complete ToS process.

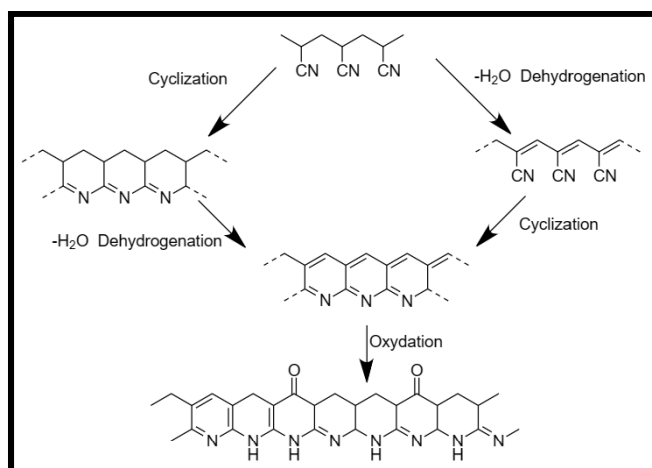


Figure 13. General reactions during Tos. First, cyclization and dehydrogenation reactions happened. Second, oxidation of the PAN structure occurs to form ladder-like PAN nanofibers. Inspired from reference [67].

- Functionalization of PAN fibers

Functionalization of nanofibers may enhance some properties or even bring new ones. For example, collagen was deposited on PAN and PLGA scaffold by LBL technique. This improved attachment and spreading of mouse lung fibroblast [88]. In another study, Wahab et al. used titania anchored with silver nanoparticles for antibacterial applications. Silver nanoparticles are effective

and efficient alternatives to conventional antimicrobial materials, effective against a wide range of bacteria, viruses, and fungal species. The composite nanoparticles were synthesized by an environmentally green approach using dopamine hydrochloride, which was utilized as an adhesive to form adhesive coating on titania surface reducing the silver ions into Ag nanoparticles. The Ti/Ag nanoparticles (5 and 10 wt%) were added in a DMF solution and then sonicated for one hour, to make a stable colloidal solution. Then PAN was added and stirred for 24h. Nanofiber composites were electrospun with needle tip to collector distance and voltage of 15 cm and 15 kV respectively. The nanocomposites showed regular and bead free nanofibers, and an increase of fiber diameter was observed while using nanoparticles. This has been interpreted as resulting from the localized agglomeration of nanoparticles within the fibers [71].

Engel et al. used gold salt to functionalize nanofibers for catalytic applications. They mixed HAuCl_4 and DMF at 70°C and then added PAN. The mixture was stirred for 3 hours at 70°C and then electrospun. They demonstrated that PAN act as a reducing agent and is able to reduce gold salt under soft condition (70°C) to create nanoparticles, contrary to other polymers, which need extra energy, such as reflux conditions, microwave heating, or UV-irradiation for nanoparticles growth [69].

In one study, Patel et al. used hydrothermal method, by electrospinning a PAN solution containing $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ and AgNO_3 precursors. The PAN/ $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}/\text{AgNO}_3$ obtained membrane was mixed with $(\text{NH}_4)_2\text{CO}_3$ and transferred into an autoclave for hydrothermal reaction, at 150°C for 3 hours. PAN/ ZnO-Ag composite nanofibers membrane was obtained, with good antibacterial properties [72].

Finally, Zhang et al. prepared PAN fibers modified with amidoxime function, through the treatment of the mats in hydroxylamine (NH_2OH) aqueous solution. The $-\text{C}\equiv\text{N}$ groups on the surface of the PAN fibers react with NH_2OH molecules and lead to the formation of $-\text{C}(\text{NH}_2)=\text{N-OH}$ groups (also called amidoxime), which were used for coordination of Ag^+ ions. It is well known that nitrile groups can be chemically converted into amidoxime groups, which can coordinate with a wide range of metal ions, including silver. Subsequently, the coordinated Ag^+ ions were converted into silver nanoparticles with size being tens of nanometers [73]. Sirelkhatim et al. used these membranes to study the antibacterial effects of amidoxime-Ag nanoparticles on bacteria and fungus, to better understand the interactions between membrane and bacteria [12].

3.2.3. Peptides and proteins

When peptides and proteins are electrospun, many points must be considered before. Degradation and denaturation are impacted by temperature, humidity, pH, solvent... Even if spinnability is also affected by these parameters for polymers, here is one more element to monitor: the conformation of the protein, which is essential to its bioactivity. The advantages of using these elements are their biocompatibility, their biodegradability, their isoelectric properties that facilitate drug loading and finally their structural properties that allow them to form ECM structures more easily [89]. Many peptides and proteins have been studied. First, soy protein was used for wound healing. Har et al. showed that scaffolds made with this protein enhanced reepithelialisation in preclinical tests. Then, silk fibroin presents many advantages, such as biocompatibility, softness, flexibility, brightness, mechanical properties among a lot more that make it a performant candidate. It was, for example, used for production of tubular scaffold to stimulate blood vessels regeneration [89]. One can also find electrospun scaffolds made with hemoglobin, fibrinogen, elastin, collagen, gelatin, keratin, and bovine serum albumin [89].

One strategy to have more stable scaffolds and to electrospun the solution more easily is to mix protein/peptides with a poly-mer. Another strategy is to use synthetic peptides. Studies of scaffolds using them are increasing since they can be manipulated (modification of amino acids sequence for example), used without toxic solvent and more easily spinnable [89].

3.3. Control of scaffold architecture

ECM plays a significant role in cell behavior. To better mimic this environment, it is necessary to produce scaffold with specific surface properties and an accurate architecture. Scaffold surface can be modified by chemical treatment and its structure can be controlled and modulated through different structural approaches.

3.3.1. Modification of the surface

Surface has a significant impact on cells since their interactions influence activation of signaling pathways and thus migration, differentiation, and proliferation. Thus, surface morphology and potential as well as the functional groups present at the surface must be well controlled to fully understand the mechanisms implied.

- Biochemical modification

Incorporating biomolecules or nanoparticles into electrospun nanofibers brings additional functions and enhanced performances. The biomolecules can be proteins, peptides, or plant extracts for example. The nanoparticles can be made of metals [71], metal oxides [13,91], metal salts [70], graphite [91] or even one-dimensional (1D) nanostructures such as carbon nanotubes [92], etc. The commonly used nanoscale component includes nanoparticles made of Ag [12], Au [70], Zn [13] and Ti [71].

Two strategies, not necessarily mutually exclusive, can be employed to modify scaffold surface [60].

The first strategy is to incorporate biomolecules within the initial solution. The electrospinnability of such a formulation depends on the type, the size, and the concentration of the added component, which will also affect the morphology and properties of the resultant electrospun nanofibers. A stable dispersion of the nanoscale component in the polymer solution is necessary to obtain homogeneous distribution of the nanoscale components [34]. For example, functionalization of PCL nanofibers with galactose biomolecules improved secretion of collagen and glycosaminoglycan as shown by Gopinathan et al [60]. Another example is silver nanoparticles added to MADON nanofibers to induce antibacterial properties that were not observed in pure MADON scaffolds [93]. In another study, Wehlage et al. [13] investigate the nanomaterial composite PAN/Maltodextrin/Pea Protein (PAN/MP) and PAN/Casein/Gelatin (PAN/CG) in order to improve cell adhesion on PAN. Blending PAN with additives improves cell bonding. All solutions were prepared by stirring PAN with casein/gelatin and maltodextrin/pea protein during 2 hours at room temperature. Afterwards they were sterilized by autoclaving for 20 min at 121 °C, to promote cell growth. These blends were found to result in larger fiber diameters (around 600 nm) than pure PAN nanofiber mats. It appears that the preparation process, autoclaving among other, influenced PAN/CG nanofiber morphology by denaturing protein and melting gelatin during heat treatment likely. Nevertheless, they enabled cell growth and adhesion. The best cell proliferation and adhesion, however, was found in PAN/CG nano-fiber mats.

The second strategy is surface modification after electrospinning by adding chemically and/or physically bioactive molecules and ligands. Plasma treatment introduces polar functional groups that enhance wettability, polarity, protein adsorption and improve cells behavior. For example, capture of porcine mesenchymal stem cells on PLLA nanofibers was improved after oxygen plasma treatment [94]. Coating is another way to modify surfaces introducing hydrogen bonding, Van der Waals interactions and π - π stacking [60]. As instance, it was shown that, by coating PDA on PLA nanofibers, hADSCs adhesion was promoted [95]. Also, Electrostatic Template-Assisted Deposition is a new method allowing the spatially controlled deposition of biomolecules on nanofibers electrospun. When nanofibers are on the conductive part of the collector, HA electrosprayed particles are deposited on it whereas they don't reach nanofibers on the non-conductive part of the collector [96]. Layer-by-layer (LBL) is another method based on adsorption of oppositely charged particles due to electrostatic forces, hydrophobic interaction, and covalent bonding [88].

Finally, concerning the nanoparticles, they can be generated on the surface of electrospun nanofibers through surface deposition, in situ synthesis, or hydrothermal treatment. The simplest method is to immerse the as-spun nanofibers in a colloidal suspension of nanoparticles to catch the nanoparticles through chemical or physical binding. A requirement is that the nanofibers are not soluble in the colloidal suspension [34]. A coating allows, for example, the formation of gradients to improve cell polarization or direction of migration. Karl et al. showed a preferential direction of RGC's axons towards higher concentration of Netrine-1, which is promising for disease of optical nerve [97].

- Surface morphology and potential modification

When cells are interacting with scaffold, signals are transduced to the nucleus triggering signalization cascades [92]. Recently, Metwally et al. have produced an electrospun PCL scaffold with different surface potentials and different morphologies. They could compare the impact on cells to have either porous or smooth fibres composing the scaffold. Then, by changing voltage polarity during electrospinning, they modified surface charge and zeta potential of the fibres. They showed that bone regeneration was enhanced when fibres were porous, surface potential was higher and zeta potential negative. Porous fibres promoted initial cell adhesion, collagen mineralization was improved when surface potential was higher and finally, negative zeta potential enhanced calcium mineralization [98].

3.3.2. Modification of the structure

- Alignment

Alignment is a key factor to monitor. Some tissues show natural oriented arrangements such as blood vessels [60]. As an example, Cho et al. observed that formation of myelin-like segments from induced pluripotent stem cells was promoted on well aligned PCL nanofibers [99]. Sometimes, both aligned and non-aligned fibres can be necessary. Park et al [94] created a scaffold with one layer of aligned fibres, that promoted alignment and differentiation of C2C12 myoblasts, and one layer of non-aligned fibres that gave good mechanical properties. To control alignment, mechanical treatment can be performed to force orientation of fibers. However, this method can break fibers. Thus, modification of collector shape or speed of rotation is usually the method used to modify fibers alignment [100].

- Diameter

Diameter is another factor to control. According to cell types, diameter must be changed to better mimic the microenvironment of cells and so induce in vivo cell behavior [101]. Migration, cell adhesion or proliferation are impacted by fiber diameter. For instance, 3T3 fibroblast adhesion is promoted on nanofibers of 428nm whereas larger diameter is required for platelet attachment. Also, diameter impacts gene expression and cells phenotype. For example, osteogenic genes were upregulated on nanofibers compared to microfibers [101]. Han et al showed better smooth muscle vascular cell proliferation on 500nm nanofibers compared to 1µm fibers [102].

- Porosity

*Phase separation

The introduction of pores can be induced from a biphasic medium by transforming one phase into pores and the other into a nanofibrous matrix. Separation can be achieved between polymer and solvent or polymer and non-solvent [103,104].

The first method is induced by cooling very quickly the not fully solidified jet by evaporating a very volatile solvent or by collecting the nanofibers on a cryogenic liquid. Thus, using THF, chloroform or acetone as solvents, porous

nanofibers of PMMA, PS, PCL, cellulose acetate, or poly (vinyl butyral) were obtained. Similarly, PS nanofibers with a high pore density were obtained by collecting the fibres in a liquid nitrogen bath followed by vacuum drying [105].

The second method is induced by vapor or liquid phase separation. For vapor-induced separation, electrospinning is performed under humidity and the water molecules in the air act as a

non-solvent. The solvent evaporates and the water vapor condenses into exceedingly small water droplets. Polymer-enriched and polymer-depleted domains are thus formed. The former domains solidify to become the nanofibrous matrix, and the latter forms the pores upon evaporation of water and solvent. Porous nanofibers of PVDF, poly (ether imide), PET and PS were produced in this way [105].

Another method is the collection of fibres in a non-solvent bath. The residual solvent, in contact with the non-solvent, induces phase separation and pore formation. Thus, by electrospinning a solution of PAN/DMF in an ethanol bath, porous PAN nanofibers were produced whereas solid nanofibers were produced by electrospinning the same solution in hexane [106].

*Use of a sacrificial phase

Small molecules (e.g., salt), copolymer blocks, polymers or nanoparticles can function as a sacrificial phase and be removed via leaching or calcination.

The sacrificial polymer must be water soluble. By varying the ratio of matrix polymer to sacrificial polymer, the pore size varies. PEO is one of the best candidates for the sacrificial phase [107]. In one study, porous carbon nanofibers were formed by carbonizing PAN and Nafion nanofibers. Thus, PAN was converted to carbon and Nafion was decomposed. In another study, coaxial electrospinning was used to generate porous TiO₂ nanofibers. The inner phase was PS in DMF and THF while the outer fluid was PVP in ethanol containing Ti (OiPr)₄. The two solutions partially mixed in some regions of the jet and rapid evaporation of the latter separated the two polymers. Then the continuous matrix of PVP and TiO₂ formed, with nanoscale domains of PS in it. After calcination, porous TiO₂ nanofibers were finally obtained by removing PVP and PS [108]. Sadeghi-Avalshahr A. et al. used PVP as a sacrificial fiber to increase pore size and improve cell infiltration. PCL and PVP solutions were electrospun simultaneously using two independent needles placed opposite each other. The resulting fibres were placed in water for PVP dissolution. The fibres were then grafted with collagen and chitosan. This allowed the HDF cells to infiltrate the matrix [109].

*Salt leaching

To create controlled pore size, salt particles with known size can be dispersed in the polymer solution before electrospinning and leached out after [107]. Nam et al. introduced salt particles using a sheath around the needle. The resulting PCL nanofibers exhibited a uniform network with well dispersed salt particles [110]. Kim et al. simultaneously deposited salt particles and electrospun nanofibers producing a porous network of hyaluronic acid and collagen. After salt leaching, the structure of the matrix is minimally affected (a slight shrinkage is observed) [111].

*Cryogenic electrospinning

For this method, the collector system must be kept at a low temperature to allow the formation of nanofibers and crystals and thus form a fibrous mesh containing crystals that are then removed by freeze-drying [25]. Pores are thus obtained and can vary according to the quantity of crystals formed, the size of these crystals depending on the humidity [112]. For example, NIH 3T3 fibroblasts were able to infiltrate a porous poly (D-L, lactide) matrix obtained by this method while this was not the case in a conventionally obtained matrix [113].

*Combination of nano and microfibers

This combination produces larger pores with greater interconnectivity. While nanofibers improve the cell adhesion and proliferation, microfibers have larger pore sizes that facilitate cell infiltration [114]. This type of matrix can be obtained by electro-spinning microfibers and nanofibers using simultaneously two different needles. Pham et al. thus generated a PCL matrix composed of microfibers (5 µm in diameter) and nanofibers (600nm). The large pores allowed cells to infiltrate the matrix and the nanofibers facilitated cell distribution and growth. It was shown, in this study, that stem cells differentiation was impacted [115].

*Ultrasonication

To increase pore size, ultrasonication is a possibility since it allows the scaffold to be loosened [116]. Exposure time and energy are the two parameters that influence the pore size obtained. Gu et

al. demonstrated that electrospun chitosan fibers that underwent ultrasonication allowed greater proliferation and infiltration of human dermis fibroblasts [117].

*Liquid collector bath

The obtention of large pore can be achieved using a liquid reservoir since fiber dispersion is increased and thus fiber binding decreased [107]. Yang et al. produced PLGA/PCL matrices using an ethanol bath. BMSCs infiltrated the matrix and deposited it on a cartilage-specific matrix. The matrix was then implanted subcutaneously in rats and bone formation was observed after 8 weeks [118].

It is also possible to generate yarn structure using a dynamic liquid system. A vortex of water is created that twists the nanofibers deposited on the surface of the water and forms a continuous thread. These threads can be collected on a rotating collector to form a 3D array of aligned nanowires. Xu et al. used a P(LLA-CL)/collagen matrix obtained by this method for tendon regeneration [119].

*Annealing

Annealing consists in heating a polymer above its glass transition temperature and then cooling it. Porosity, hydrophobicity, mechanical properties, and molecular structure are impacted [120]. PLA was electrospun on a rotating collector at 100 rpm to obtain an isotropic matrix. Then the matrices were placed between two glasses and heated in a muffle furnace under different annealing conditions. First, the annealing temperature was set at 90°C and the time varied (30 to 120 min) and then the time was set at 30 min and the temperature varied (90 to 105°C). As the annealing time increases, the pore size decreases due to shrinkage, fibre thickening and fibre melting. By increasing the annealing temperature, the fibres thicken, the pore size decreases, and the fibres fuse together. The annealing treatment increases the fusion between fibres and the degree of crystallinity but decreases the ductility [120].

*Influence of flow rate

Rnjak-Kovacina et al. [121] studied the impact of flow rate on the porosity of their tropoelastin scaffolds. Two different flow rates (1 and 3 ml/h) were used. They showed that fibre diameter, thickness, porosity, and pore size increased with flow rate. The cells proliferate on both matrices, but they only infiltrate the scaffold when seeded on the one obtained at 3mL/h. Also, the fibroblasts seeded on this matrix have deposited collagen and fibronectin, thus showing the capacity of the cells to proliferate and modify their environment. When the matrix was injected into rats, an early stage of angiogenesis was observed, and no negative immunological response was produced.

*Specific collectors

To influence the pore size, the deposition support of fibers can be modified [107]. For example, Blakeney et al. [122] demonstrated that the use of a spherical foam collector with stainless steel probes results in a relaxed, uncompressed, cotton ball shaped matrix. Proliferation and infiltration of INS-1 cells were improved. Another method is to send an air flow pressure, whose velocity influences the pore size by loosening electrospun fibres [107]. For example, a specific collector was created by Liang Chen et al. [123], to produce fibers with a controlled microstructure by controlling the speed of this collector. The latter collector has five microcontrollers, four micro-stepper drivers, and four collecting surfaces. The hexagonal needle produces an identical electric field at each vertex of the needle, improving the homogeneity of the formed nanofibers. They studied four different speeds. The pore size is found to increase with the speed. The authors studied the behavior of the pre-osteoblastic MC3T3-E1 and pre-osteoclastic RAW 264.7 cells that participate in bone regeneration. They were able to observe that MC3T3-E1 survived best in the matrix with the largest pore size while the RAW 264.7 survived best in the medium pore size nanofibers. The high pore size nanofibers promote the differentiation of pre-osteoblast cells.

3.4. Application in biological field

3.4.1. Studying migration.

Cells migration is decomposed into steps (polarization, actin polymerization, assembly, and disassembly of focal adhesions) leading to spatial cell asymmetry and cell body translocation [19]. Environmental chemical and physical properties are leading to a variety of signals, triggering

signalization cascade, and ultimately impacting migratory behavior: single or collective, amoeboid, or mesenchymal, focal adhesion independent or dependent, directionally persistent, or not. In contrast with single cell migration, during collective migration, cells will be influenced both by cell-cell junction (described as implicated in mechanosensing and signaling), and by extracellular matrix stiffness sensed at integrin focal adhesions or thanks to mechanosensitive ion channels, leading to modulation of the migration, depending on ECM properties.

Different kinds of nanofibers have been made up to reproduce individually or in association, different physical and chemical parameters found in vivo.

During cancer the alignment of the ECM fibers has been extensively described as modified by the tumor growth itself, the ECM remodeling by cancer cells and the imbalance between ECM protein production, degradation, and crosslinking. All those events are leading to the creation of a tumoral microenvironment promoting cell migration, called desmoplastic environment, and presenting ECM protein composition changes, fibers alignment and stiffness increase. When cancer (and healthy) cells are cultivated on desmoplastic-like nanofibers configuration, the cells migrate along the nanofibers and the migration speed is increased [19]. On the contrary on non-aligned nanofibers the cells migrate along all directions and the translocation takes place over a short distance.

The ECM composition and protein abundance have a profound impact on cells behavior and migration as well. ECM cells receptor expression is co-regulated with ECM changes observed in physiological and pathological context, and different ligand/integrins combinations are deeply changing the traction forces exerted by the cells, the adhesion formation and the following mechanosignaling [124]. Adhesion force must therefore be neither too strong nor too weak, to optimize and adapt cell migration within the microenvironment. In the case of ECM protein low abundance, or ligand/integrins combination not corresponding, cells are adapting their migrating behavior. In the publication of Saleh et al, the behavior of glioma stem cells expressing mainly collagen receptor, was compared while migrating on in vitro laminin coated nanofibers environment and after glioma stem cells xenograft into mouse brain (collagen is not abundantly naturally expressed in brain) [47]. Then GSCs appeared as migrating collectively to escapes anoikis (death by cell detachment of ECM matrix), both on nanofibers and mouse brain. 1. Consequently, coating of nanofibers with proteins is greatly affecting cell behavior and migration, modulating migration behavior both in vitro and in vivo [25,125]. Also, gradient of bioactive agents can guide migration, thanks to chemotactism, with cells preferring to migrate along the gradient towards higher ECM protein concentrations. The dependence on ECM protein or other bioactive agent for migration is shown as well by performing competition between a BSA gradient and a bioactive agent deposited on the less concentrated by electrospray, within microfluidic device or by electro hydrodynamic printing [126].

Mechanical cues as stiffness have been observed many times as influencing migration, both in 2D and 3D, and occur as well during cell migration on nanofibers with different stiffnesses. Marhuenda et al. [19] used PAN nanofibres stabilized at 250°C for two hours, with stiffnesses modulations to study glioma stem cell migration, in a 3D fibrillary environment depending on rigidity. They modified the stiffness of nanofibers by adding multiwalled carbon nanotubes (MWCNTs) in the polymeric solution before electrospinning, which modified the intrinsic young's module of PAN fibers. They have obtained and selected electrospun fibres stiffness ranges from 3 to 1260 kPa, which are values reported for human healthy tissues and gliomas [127]. It appears that the amount of MWCNTs is independent of fibers diameter, and 166 kPa stiffness fibers was found as optimal for the glioma stem cell migration, increasing migration rate by ~4 [19]. These values around 100kPa have been described previously as well as optimum for migration as well by Bangasser et al.[128]. Also, between 3 and 1260kPa, can be found a range of stiffness that can be used for other cell subtype in order to make cell culture in a relevant environment in terms of stiffness [129].

3.4.2. Cancer research

Whereas 2D scaffolds for cancer study are routinely used, it is well known that the results obtained are far from reality and can lead to artefact. Cancer tissue engineering in 3D nanofibers

scaffolds better mimics the reality than 2D scaffolds [130] and focus is currently on reducing animal model thanks to in vitro pertinent 3D matrices modelling cancer microenvironment. These scaffolds are used to study drugs delivery, to isolate CTCs, to study modifications of cells behavior due to chemical or physical changes of the environment. One of the differences between 2D and 3D scaffolds is the access to nutrients. In 2D scaffolds, nutrients are uniformly spread while spatial gradient is observed in vivo, especially in for cancer cells due to the rigidification of the tumorous environment [45].

Nanofibers materials are produced with properties mimicking as closely as possible to the ones found in tumors environment and aim to reproduce ECM interactions when cultivated on fibers. It is well known that cancer cells proliferation and migration are impacted by ECM's stiffness and ECM composition, [131–133] as well as the tumor grade, aggressivity and drug resistance. Chitosan/poly (ethylene oxide) nanofibers scaffold has showed remarkable results as well. When MCF-7 breast cancer cells were seeded on it, 3D breast cancer tissues were formed after 10 days with higher proliferation rate than what observed in conventional 2D adherent plates [134].

In another study, Saha et al. produced PCL electrospun scaffolds with either aligned or non-aligned fibers. The MCF-7 cells seeded on them shown morphologies different in the two environments (elongated shape and flat stellar shape respectively) highlighting topography of the fibers has having a great impact on tumor cells behavior [135]. Ricci and al. showed that PDAC cells (pancreatic ductal adenocarcinoma) had different behavior depending on their seeding on nanofibers scaffolds or sponge scaffold. In the case of PDAC, spongy scaffold seems to increase proliferation compared to fibrous scaffold. The secretion of matrix metalloproteinase (MMP) appeared as changing regarding the material composition of the sponges (poly (vinyl alcohol)/gelatin (PVA/G) mixture and poly (ethylene oxide terephthalate)/poly (butylene terephthalate) (PEOT/PBT) copolymer but was secreted in both sponges and fibers [136]. Kim et al. were able to coculture BM-DC (bone marrow dendritic cells) and CT26 (colon cancer cells) and reproduce the crosstalk in vivo happening in between those cells. BM-DC chosen as immune cell model and presenting antigens to T cells, could swallow up mitoxantrone-treated CT26 cancer cells as it would have been the case in vivo. Those coculture could then provide wonderful in vitro model of engulfment of cancer cells by T cells [137]. Jain et al proved that glioblastoma cells (U87) migration was promoted on aligned fibers scaffolds [138]. This enhancement of migration could be used to guide glioblastoma cells away from the primary tumor trap thanks to topo induction and attract them in an extracortical trap where it would be more accessible and thus enable an efficient operation.

Primary culture of human glioma stem cell (GSC) when deposited on poly acrylonitrile nanofibers (PAN) by A. Saleh and E. Marhuenda et al., appeared as capable of modulating their migrating behavior. Those migrations modulations, individual or collective, were depending on the presence of laminin coating on the surface, of the ECM receptors expression of the GSC, and were recapitulating the migration behavior observed in vivo during xenograft of those same GSCs. As well the transcriptomic assay performed in 3D compared to classic 2D exhibit 98 genes deregulated implicated into cell cycle, proliferation and kinesin family suggesting a shift to an aggressive and invasive phenotype [47].

E. Marhuenda et al. investigated the migration of GSCs deposited on 3D PAN nanofibers, depending on the microenvironment stiffness. The stiffness of the nanofibers has been tuned through the addition of MWCNTs, from 6 to 1260kPa. The GSCs were found to migrate 4 times more at 166kPa than in the other stiffnesses and were associated with higher invasiveness markers. This exhibits the existence of optimal stiffness for migration within 3D nanofibers mats, and even appeared as able to trigger migration by itself in proliferation conditions. This optimal stiffness is very likely depending on cell type [19].

Another possibility to use nanofibers is immunosensing for cancer screening. It is a way to detect tumor markers or circulating tumor cells in blood at an early stage of cancer development [25]. By functionalizing nanofibers with bioactive molecules (such as enzymes, antibodies), specific cells can recognize it and adhere. Ali et al. created a zinc oxide electrospun nanofibers scaffold decorated with

EGFR-2 allowing detection of breast cancer biomarker for early diagnostics. This system, based on electrochemical impedance technique, detects 1fM concentration fast [139].

Another example is a scaffold made of ZnO nanofibers containing MWCNTs and conjugated with the anti-carcinoma antigen-125 antibody, carcinoma antigen-125 is a specific marker for ovarian cancer [140]. Similarly, an oxygen sensor was created by combining PCL nanofibers with oxygen-sensitive ruthenium [141].

The capture of CTCs could allow to slow down and prevent metastasis, as well as obtaining information to better diagnose cancer and analyses the effectiveness of drugs. CTCs (HeLa, KB, A549, and MCF-7 cells) were specifically captured in a microfluidic chamber by PLGA nanofiber arrays coated with hyaluronic acid (CD44 receptors for hyaluronic acid are overexpressed in many cancers) in the study of G. Xu et al. [142]. The HeLa captured cells continued to grow on the nanofibrous membranes in the micro-fluidic chip without compromising cell viability making it promising for individualized medicine research. By capturing CTCs, drugs could be assessed and monitored in real time.

pH plays a central role in many biological processes and particularly in cancer. To quantify and perceive this biological parameter, organic hybrid electrospun nanofibers have been filled with pH sensing capsules. They changed optically when submitted to proton-induced switching. This change was analyzed with fluorescence detectors and led to quantify of local proton concentration. It would be interesting to use this kind of pH sensing electrospun fibers to measure in vivo, both spatially and temporally, the extra-cellular pH to study glycolysis (inhibiting anti-cancer drugs) [143].

3.4.3. Biosensors

Biosensors are essential for the detection, quantification and monitoring of analytes presents to provide an accurate and performant treatment [144]. First, electrospun nanofibers have been used to detect DNA. By electrospinning cellulose monoacetate and tetraethyl orthosilicate, DNA molecules were able to be captured on the surface of fibers. This allowed then to monitor guanine-base oxidation in single strand DNA electrochemically [144]. Another electrochemical biosensor was created to detect early-stage of Alzheimer's disease. By functionalizing carbon electrode with Tin oxide electrospun nanofibers, the hormone-specific β -Amyloid was immobilized and could be titrated. Its absorption on the nanofibers induced a reduction of conductivity [144].

Hydrogen peroxide detection is of great interest, especially for early-stage cancer diagnosis. Daemi et al. used a ZnO-CuO hybrid nanofiber to detect H₂O₂ by analyzing charge transfer resistance and electrocatalytic performance [144].

Electrospun nanofibers can be used as well as sensors of glucose and PAN nanofibers can be used to produce glucose biosensors. PAA/PAN/AuNPs electrospun nanofibers allowed the monitoring of glucose level with a better sensitivity than the screen-printed carbon electrode by itself [145]. Highly sensitive and long-term stable glucose sensors have been fabricated by encapsulating enzymes into ZIF-8/CA nanofibers, then coated with MWCNT and AuNPs to form an electrode [144].

3.4.4. Stem cells differentiation and cell therapy

Stem cells are promising for new medical treatments and regenerative medicine offers great hopes in repairs response after injuries or diseases. Stem cells or precursors cells can differentiate into many cell types depending on their physical and chemical microenvironment. To regulate the proliferation and differentiation of stem cells, matrices are made to mimic stem cell niches. Gooraninejad et al. [146] used PAN nanofibers scaffolds as support for cell therapy by allowing the proliferation and differentiation of stem cells. They seed human endometrial cells on PAN nanofibers, inducing their differentiation into PDX1-expressing cells, before and then transplanted it into diabetic rats. They showed that both weight and blood glucose in diabetic rats were decreased. This would require further investigations. Even if more studies are necessary, this could be an open promising treatment given to opportunities of innovations for diabetics. As mentioned previously, physical parameters of the microenvironment, such as surface topography, dimension, and mechanical

properties, can impact cell differentiation. MSC-derived iPSC cells differentiate better on aligned nanofibers [147].

Mechanical properties have a particularly important impact. For example, by reducing the matrix thickness of gelatin and Tecophilic nanofibers, SMCs (smooth muscle cells) differentiate into contractile phenotype and SMC proliferation is reduced.

Finally, biological signals such as growth factors, proteins and peptides can be incorporated into the matrix to direct stem cell differentiation. For example, adipose cells derived from MSC were co-cultured with human tenocytes and umbilical cord endothelial cells on a scaffold made of PCL and PLA interlaced nanofibers. The cells expressed higher activation of tendon-associated markers than on PCL nanofibers alone. A matrix fabricated by electrospinning serum albumin and doped with hemin and then functionalized with proteins and growth factors has a favorable microenvironment, guidance topography, bioactive molecules, and potential electrical stimulation. This matrix has been shown to be effective in promoting the differentiation of iPSC cells into neurons.

However, stem cell culture in a biocompatible scaffold, promoting their differentiation preceding transplantation remains a challenge.

3.4.5. Tissue engineering

A scaffold for tissue engineering is a structure made to facilitate growth, proliferation, and differentiation of cells, works as a framework for tissue formation and can be implanted into a patient. The creation of tissue by seeding cells on electrospun nanofibers has already been evaluated for bone regeneration, neuroscience, and skin reconstruction.

For tissue regeneration, growth factors are required but are unstable and have a short half-life. Their incorporation into nanofibers would allow them to maintain their activity and to stabilize them. Promising results have been obtained with fibroblast (FGF), epidermal (EGF), transforming (TGF), neural (NGF), platelet (PDGF), connective tissue (CTGF), vascular endothelial (VEGF) growth factors [148]. Qu et al (2019) encapsulated BSA and transforming growth factors (TGF B3) in PCL/PLGA nanofibers. This matrix could then induce the differentiation of synovium stem cells into fibrocartilage tissue [149].

The creation of Shape Memory Polymer (SMP) nanofibers is interesting for tissue engineering specifically to minimize invasive surgery [150] and magnetic nanofibers is also of interest as, magnetic stimulation allows the alignment of cells on a magnetic matrix [151].

Wieringa et al. were able to show the effect of synergistic action of different peptides (P20 and RGD among others) on neurite and glia growth [152]. This is illustrating the interest in functionalizing heterogeneous scaffolds allowing to study cell behavior in more accurate models of the ECM and better understand the complexity of the biological system in which cells evolve.

- Bone tissue engineering

Nanofibers scaffold offers a more optimized environment than other 3D cell culture system for cell attachment, osteoblastic differentiation, mineralization, and better bone regeneration [61]. Electrospinning allows to customize scaffold to provide them the appropriate mechanical properties wanted and high porosity [153]. Wang et al. for example used PLLA solution incorporated with PHBV to be electrospun for bone tissue engineering. The combination of those two polymers were offering shape memory property and good mechanical properties respectively [154]. Another study presented PAN/nCB/HA electrospun nanofiber, an excellent candidate for bone and other hard tissue engineering. HA was used to better mimic natural bone environment and nCB (carbon black nanoparticle) improved mechanical properties of PAN/HA scaffold. The full combination enhanced MC3T3-E1 osteoblast cells proliferation and adhesion [155].

To promote regeneration of tissues, matrices often combine a biocompatible polymer with an inorganic phase [25]. Matrix of PLA, PCL and PEO fibers contains large, interconnected pores and has been shown to allow osteogenic differentiation of MSCs. The addition of minerals and bioactive agents enhance the ability of the scaffold to promote differentiation.

Some biomolecules can be added to promote cell activation and cell growth. For example, Rachmiel et al. had electro-spun a solution of PCL and hyaluronic acid (HA) which led to enhance

osteogenic differentiation. More recently, PA6/CS electro-spun scaffold was obtained and functionalized with HA particles. PA6 is mimicking collagen and biodegradation rate of CS, present in ECM, is quick enough to ensure new bone formation. Finally, HA, which was mineralized, enhancing MC3T3-E1 cell attachment and proliferation. All these results make it a promising candidate for bone tissue engineering. The functionalization of PCL electrospun nanofibers with E7 and BMP-2, two mimetic peptides found in native bone ECM, promoted BM-MSC adhesion and osteogenic differentiation. Also, it was shown that PAN nanofibers scaffold, with Ag particles, allows osteoblasts to survive and proliferate. Ag biocompatibility, antibacterial property, and ability to be sterilized make it a suitable candidate for a prosthesis with double function (one side for bone regeneration and the other side for soft tissue repair).

Plant extracts, essential oils and other phytoconstituents (such as Lemongrass, Baicalein, or cinnamon) have been used to treat diseases, for their properties helping the reparation and/or regeneration hard tissues for many centuries [156]. Electrospinning of these constituents allows exploiting their potential. For example, Panax ginseng incorporated in PCL nanofibers improved proliferation of mesenchymal stem cells and induced osteogenic gene expression [156]. Curcumin is also known for its benefits in bone regeneration.

One main property of bone tissue is piezoelectricity because of collagen micelles [153], inducing bone growth and regeneration. Kitsara et al. produced a scaffold made of PVDF electrospun nanofibers treated with oxygen plasma. Voltage-gated calcium channels were stimulated, then promoting cells activation [154]. Carbon nanofiber matrices, made by electrospinning PAN followed by calcination and carbonization, showed a promising result for the stimulation of bone tissue regeneration by applying an electric current as well. MG-63 cells were cultivated onto those matrices and an electric current was sent, concentrating into the highly conductive CNFs. The stimulation of the cells through the CNFs activating their voltage gated sodium channels changing the transmembrane potential of the MG-63 cells. This electrical stimulation of cell promoted proliferation and the ALP activity (alkaline phosphatase) of bone cells [157]. The same group showed the functionalization of those matrices with hydroxyapatite crystals was promoting cell growth and differentiation of bone cells as well. These bio ceramics were mimicking the mechanical properties of the bone, were acting as a reservoir of calcium and phosphate, and was promoting osteoconduction as well as osteoinduction [157].

Finally, the topographic structure is an essential factor to study [153]. For example, Xu et al. [158] produced a scaffold by electrospinning PLA and Chitosan and by modifying the temperature of the jets, they could form chitosan islands structure at the surface that then offer a roughness and a balance between hydrophilicity and hydrophobicity for cell adhesion and recognition site. A high degree of roughness favors the expression of osteogenic genes while a low degree of roughness favors the chondro-genic differentiation of mesenchymal stromal cells [159].

- Neural tissue engineering

In the context for neural tissue engineering, nanofibers scaffolds are improving nerve regeneration [61].

Spinal cord, nerve, and brain injury as well as neurodegenerative diseases are characterized by alteration of the architecture of the tissue leading to growth inhibition and axon guidance as well as glial scar [160]. Neural tissue engineering is important since it is difficult to find donors for the treatment of peripheral nerve injuries [60]. Electrospinning offers the possibility to build scaffolds with mechanical and biochemical properties inducing differentiation of neural stem cells [161]. Lu-Chen et al. showed an electrospun POMA (poly(o-methoxy aniline)) fiber matrix allowing neural stem cells (NSCs) to grow and that could be used for neuroscience research and tissue creation [45]. In another study, Jenab et al. produced a scaffold by electrospinning a solution of PAN and Kefiran (a polysaccharide produced by microorganisms) [162]. They showed an enhancement of PC12 cells viability and improvement of differentiation making it a promising candidate for neural stem cell culture and spinal cord repairing. This scaffold showed anti-cancerous properties, inhibiting MCF-7 cells growth, and promoted PBMC cells growth. More recently, PCL/PGS electrospun fibers filled with HA particles were studied. This scaffold, while enhancing cell viability and adhesion, provides

appropriate properties for nerve tissue engineering [163]. Xue et al. investigated the impact of alignment, diameter, and surface properties of electrospun PCL fibers on BMSC differentiation into Schwann cells [164]. The alignment of fibers thick enough was promoting the differentiation of BMSCs into Schwann cells and determines the alignment of these cells. The topo induction effect of this alignment appears as a promising method to act on the organization of the axon network helping axon guidance for tissue engineering.

Addition of bioactive molecules can enhance regeneration. In the same study from Xue et al., the surface coating with laminin improves the maturation and secretion of neurotrophin from Schwann cells. Those secretions help to guide and enhance the neurite extension of PC12 and DRG cells co-seeded with Schwann cells [164]. It is possible as well to produce matrices composed with different layers with varied compositions. To obtain a system with controlled distribution of PDGF, BDNF and NT-3, three layers of nanofibers were synthesized: one layer of aligned PCL nanofibers and two layers of PGLA [165]. The two layers of PGLA had a different concentration to allow a sequential release of growth factors. The PCL layer helped cell attachment and collagen type II production. Similarly, Pan et al. encapsulated insulin growth factors (IGF 1) and brain-derived neurotrophic factors (BDNF) in graphene oxide and PLGA nanofibers [166]. The in vitro results showed the protective ability of neural stem cells from H2O2-induced oxidative stress. The animal model for spinal cord injury showed an increase in the number of neurons at the lesion site and functional recovery, highlighting an improvement in nerve regeneration. Recently, Ni nanoparticles were added to PAN/PANI electrospun nanofibers to enhance conductivity and hydrophilicity of the scaffold. Thanks to this addition, Schwann cells proliferation rate was increased (2.1 times) under electrical stimulation, which revealing potential to accelerate nerve healing. Electrical conductivity is essential to ensure nerve repair and it can be modulated by the presence of Ni nanoparticles [167].

The addition of electrically conductive nanoparticles, such as CNT, electrical stimulation can be done and promote axon growth and differentiation into neurons [60]. PPy-Gr/PVA nanofibers thanks to good conductivity enhanced cell length and anti-aging effects on PC12 neuroplastic cells from adrenal gland under electrical stimulation [168]. In another study, PCL nanofibers coated with PPy form shell-sheath nanofibers and electrical stimulation, promotes neurite extension both on aligned and non-aligned nanofibers [169]. Neurites extension from PC12 cells as well as the growth of Schwann cells can be promoted as well by electrical stimulation and growth factors when cultivated on PANi, PLCL and silk fibroin nanofibers containing nerve growth factors [170].

To better mimic the spatial structure of the ECM in the nervous system, multitubular conduits should be favored. Artificial channels can be introduced into the nerve conduit to construct an artificial multichannel nerve tract, helping axon guidance and growth in the context of nerve injury. For example, PTFE rods and sucrose fibers have been used to produce channels of controlled sizes by removing the matrices or dissolving the sucrose fibers after structuring the artificial "nerve conduits". Huang et al. developed a matrix composed of an electrospun porous PCL sheath surrounding an oriented collagen/chitosan multichannel filler material to promote nerve regeneration. The filler material is obtained by a directional freezing technique using liquid nitrogen and the PCL shell by electrospinning on a steel rod to obtain a hollow tubular sheath. In vitro and in vivo studies have shown this matrix as promoting axon regeneration and stem cell migration. The results show this matrix has had the same long-term effects as autografts.

Hsu et al. fabricated a fibrous BSA matrix doped with hemin. The doping was increasing the adsorption of laminin on the fibers, essential for cell attachment, and was maintaining the coating. The hemin doping was improving the attachment and viability of hiPSC-derived NSCs. Also, on those matrixes the differentiation and proliferation were promoted thanks to the delivery of the growth factor FGF2 by the matrix. By applying electrical stimulation through the scaffold, it has resulted in neurite branches formation and enhanced differentiation.

As said in the introduction of this part, SPM can be created via electrospinning. It has been studied a lot for tissue engineering to prevent invasive surgery. For instance, Wang et al. produced a SPM scaffold that takes the form of nerve conduit only when implanted into the body. It was initially

a planar 3D scaffold to allow a better cell loading and, when implanted in the body of rats, the temperature triggered the formation of tubular conduit (Figure 7) [171].

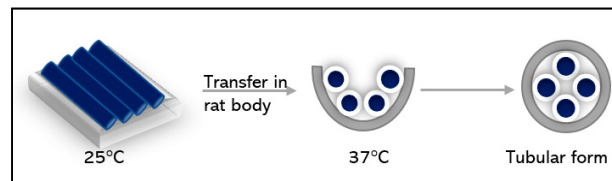


Figure 7. Shape Memory Polymer used to form a tubular nanofibrous scaffold mimicking nerve conduit. Inspired from reference [171].

- Vascular tissue engineering [60]

Electrospinning enables to obtain a controlled scaffold with an alignment that mimics the organization of smooth muscle cells and endothelial cells. One of the current strategies for integrating an artificial vascular network into organs and tissue engineered construct but it remains challenging, and many parameters must be additionally considered. The blood vessels are composed by three layers with different functions: the intima, the media and the adventitia with a layer of endothelial cells lining the blood vessels, regulating exchanges between blood stream tissues. Many vascular matrices have been produced so far. Among them, a tubular structure with a multi-layered wall mimicking multi-layered blood vessel. Also, Yu et al. produced a bilayer vascular graft with an inner layer composed of aligned TPU/SF fibers and a random outer layer composed of the same fibres.

To promote the endothelial formation and modulate endothelial cell proliferation by separately activating or deactivating the expression of specific genes, growth factors have been incorporated into the nanofibers and targeted delivery systems have been developed to deliver miRNAs locally. Tubular matrices with a degradation rate proportional to the rate of tissue remodeling have been produced to promote rapid and efficient endothelialization. PCL and polydioxanone matrices were tested in vivo in rats and the degradation of polydioxane provided sufficient space for cell infiltration. Vascular smooth muscle regeneration appeared improved. Another tubular scaffold made of SF electrospun fibers was implanted in mouse abdominal aorta and the authors could observe growth and regeneration of vascular smooth muscle as well. Liu et al. produced a sulphated SF electrospun scaffold and showed an enhancement of the adhesion and proliferation of endothelial and smooth muscle cells while promoting anticoagulation properties. However, really few electrospun scaffold is currently used as a specific vascularized organ model in vitro.

- Cartilage tissue engineering

Cartilage tissue engineering is challenging since the organisation of cartilage tissue is complex, and the chondrocytes properties differ with the region as well as the ECM composition [60]. A matrix based on gelatin and PLA nanofibers, in which hyaluronic acid was incorporated, making possible to repair a cartilage defect in rat model [172]. This porous matrix has compressive strength, superabsorbent and shape recovery properties and hyaluronic acid is a recognition site for cells being a major element of the cartilage ECM.

- Tendon/ligament tissue engineering

Tendon and ligament tissues engineering appear as promising for injury repair, as tendons have poor healing capacities. Collagen fiber bundles are easily reproducible by electrospinning nanofibers. By coating nanofibers with a gradient of platelet-derived growth factor, tenocytes markers expression was enhanced, indicating a better tenocytes differentiation from the AD-SCs [173]. The alignment of the nanofibers plays a significant role in tenocyte differentiation, as well as stiffness [173–176].

HDAC suppression plays a key role in enhancing tenogenesis on an aligned topography. Zhang et al. [176] investigated the impact of incorporating epigenetic bioactive TSA compound (HDAC inhibitor) into an aligned PLLA nanofiber for tendon regeneration. The release of TSA follows three steps: an abrupt release, one slowing down gradually, and finally a constant release. After 72h of incubation, the amount of TSA released was higher for the unaligned fibers. The aligned nanofibers

containing TSA were having an additive effect on promoting tendon regeneration. The presence of TSA allowed the formation of larger collagen fibrils and a bimodal distribution of the collagen diameter, as it can be found in natural tendon.

- Cardiac tissue engineering

Among all the matrices realized, the matrix structured with honeycombs presents the highest viability of cardiomyocytes, the deepest cellular penetration and the highest expression of genes related to the heart.

Commonly, the fibers scaffold used for cardiac engineering are either made of natural polymer, or synthetic ones like PLA (polylactic acid), polylactic-co-glycolide (PLGA), or polycaprolactone (PCL). PGS-PCL fibers functionalized with vascular endothelial growth factor (VEGF) [177] and construct with aligned and electroconductive fibers made from gelatin, PLGA and polypyrrole [178], have been used to create cardiac patch, as well as PLGA fibers coated with adhesive peptides [179].

When the surface of matrices is structured, it promotes specific stem cell differentiation and maturation as observe for iPSC cells derived cardiomyocytes during cell culture on a monolayer of gelatin nanofibers with honeycomb compartments [180]. As well, thanks to electrical polarization can be incorporated to regulate differentiation. Cardiovascular disease-specific iPSCs were seeded onto aligned PANi and polyetersulfone nanofibers. By applying electrical pulses to mimic the simulation found in the heart, the iPSCs differentiated into cardiomyocytes [181].

Helical, coiled, or spring-like fibers have been studied as well, as better representing the coiled perimysial fibers within the heart walls and helping contraction [182].

Those coiled fiber arrays containing gold nanoparticles allowed organized cell growth along the fibers and strong actinin striation promoting strong contraction force and high contraction rate [182].

Multilayered matrix has been developed as well for cardiac tissue engineering [183]. A first grooved layer was created to promote the organization of cells into contractable tissue, a second layer with cages and channels was added to organize endothelial cells into vasculatures, and a third cage-like layer was designed to encapsulate dexamethasone contained in PLGA microparticles to release this anti-inflammatory molecule in a controlled manner. These three layers were prepared separately and then embedded in a biological ECM glue to form a 3D vascularized heart tissue prior to in vivo transplantation. After implantation in rats, blood vessels containing blood cells were able to infiltrate the matrix.

More recently, a PCL/Ge/PAni scaffold was produced by electrospinning and allowed cell proliferation while preserving cardiomyocytes. Modulation of the electrophysiological properties of cardiomyocytes was possible due to the presence of PANi. This scaffold is thus an appropriate candidate for cardiac tissue engineering [184].

- Other tissue engineering

For the regeneration of tissues such as the ureter, trachea or other, the use of electrospun nanofiber matrices could be considered [25].

PLCL nanofiber incorporating ethylene diamine tetra acetic acid and sodium cholate were used to coat metal stents [185]. This has allowed to inhibit biliary tract occlusions. So far, only a few polyurethane nanofiber matrices have been assessed for the bile duct.

The ureter is easily infected and obstructed after surgery [186]. A matrix of PCL and PLGA nanofibers ureteral stent was inserted into pig ureter to evaluate how they can prevent obstructions compared to the commercial stent. They show better biocompatibility than the commercial polyurethane stent and did not induce obstruction.

Finally, preliminary studies have been conducted to regenerate the trachea [187]. A bilayer matrix of PLCL and collagen nanofibers (a porous layer on the outside and a dense layer on the inside) was constructed. Tracheal epithelial cells and chondrocytes were seeded separately in the inner and outer layer and then packed in a rat tracheal strip. They resulted in improved epithelial production, cartilage maturation, and capillary neogenesis after implantation into the rat trachea.

3.4.6. Drug delivery

Electrospun fibers are great candidates for drug delivery. They present a high surface area to volume ratio and can allow local drug delivery [61]. This method reduces drug toxicity and increases therapeutic efficacy by prolongating the dissolution rate of the drugs and make continuous drug release. It can be used to manipulate cancer cell migration and locally control the release of anti-tumors that can be loaded in fibers since they are porous structures.

Different strategies can be employed.

First, drug can be mixed in polymer solution before electrospinning [61,153]. By loading anti-drug cancer within the fibers, instability, and adverse effects on surrounding healthy tissues are reduced and local concentration in tumor sites is increased [61]. Liu et al developed doxorubicin hydrochloride loaded in electrospun PLLA nanofibers system to treat locally liver cancer and prevent post-surgery metastasis. The drug was entirely diffused from the system [61,62]. Curcumin was incorporated in PLLA/PHB solution before electrospinning. In vitro studies showed that it was released for 14 days and improved osteogenic differentiation of human adipose-derived stem cells. In another study, an RNAi and polyethylenimine plasmid (to specifically suppress matrix metalloproteinase-2 expression) and paclitaxel (a cytotoxic drug) were encapsulated in a PLGA nanofiber matrix to release both agents and thereby inhibit invasion, angiogenesis, growth, and cell proliferation [188].

The main issue for such an application is about the release and degradation rate who need to be deeply investigated to control drug release (by diffusion or diffusion and degradation of the scaffold). To prevent an initial burst release, one strategy is to incorporate a nanocarrier into the fibres [61,153]. For example, Jonah et al. [189] generated a superhydrophobic electrospun nanofiber mesh to slow down the delivery of the encapsulated lipophilic anticancer agent, 7-ethyl-10-hydroxycamptothecin. In vitro results and results from the in vivo mouse model of lung cancer suggest that this system provides controlled and specific delivery to treat glioblastoma and prevent locoregional recurrence.

Drugs can also be immobilized on electrospun fibers surfaces chemically or physically [61]. Thus, immobilization prevents drug exposure to harsh solvent or to high voltage that can denature or deactivate it. It also facilitates the process since usually drugs have high molecular weight and/or charges on their surfaces that make it more difficult to dissolve and electrospun. To optimize the drugs immobilization, fibers surfaces are first treated chemically (addition of functional groups such as hydroxyl groups). Chemical functionalization allows the control of the amount of drug immobilized and reduces initial burst release. Im and al. reduced an initial burst release by fluorinated cross-linked hydrogel fibers before adding the drug [61]. Also, a bandage was developed from electrospun PAN nanofibers in which Munaweera et al. [190] incorporated holmium-166 to cure skin cancer with-out affecting healthy cells.

Another strategy is coaxial electrospinning. The polymer shell would then protect the core filled with biomolecules [61]. For example, nanogels sensible to redox reaction were incorporated into the outer shell of the coaxial nanofibers containing BMP-2. When desulphated bonds of nanogel were modified by GSH concentration, BMP-2 was then released [191].

Also, to have a multidrug system delivery, multilayer stacked nanofibers can be produced. For example, Okuda and al. produced tetra-layered nanofiber system to have dual release: the first and third layers were drug-loaded while the second and the fourth were barrier meshes. They were able to have dual release in time by modifying barrier meshes (such as thickness) [61].

Smart polymers enable an on-demand drug delivery. For example, Kim and al. [192] electrospun a solution composed of a chemically cross linkable temperature-responsive polymer, magnetic nanoparticles, and doxorubicin to target skin cancer cells. When the nanofibers were submitted to magnetic field, the nanoparticles produced heat that led to the deswelling of polymers and thus the drug release. Also, lauric and stearic acids are phase change materials (PCM) with a melting point of 39°C [193]. Therefore, they can be used for controlled drug release by photo thermal heating. Xue et al. [193] formed lipoproteins from these acids to encapsulate NGFs. These microparticles were then electrosprayed between two layers of electrospun PCL nanofibers. Nerve growth factors, released in a controlled manner, stimulated the PC12 growth of neurites into spheroids. Another strategy is to

use pH stimulation. Cancer environment is known to have a rather acid pH (6.8). Thus, Zhao and al. [194] produced PLA nanofibers containing drug-loaded silica nanoparticles functionalized with CaCO_3 . (Figure 8) When environment was acid, the physiological pH was reduced and CaCO_3 dissolved which allowed drug release.

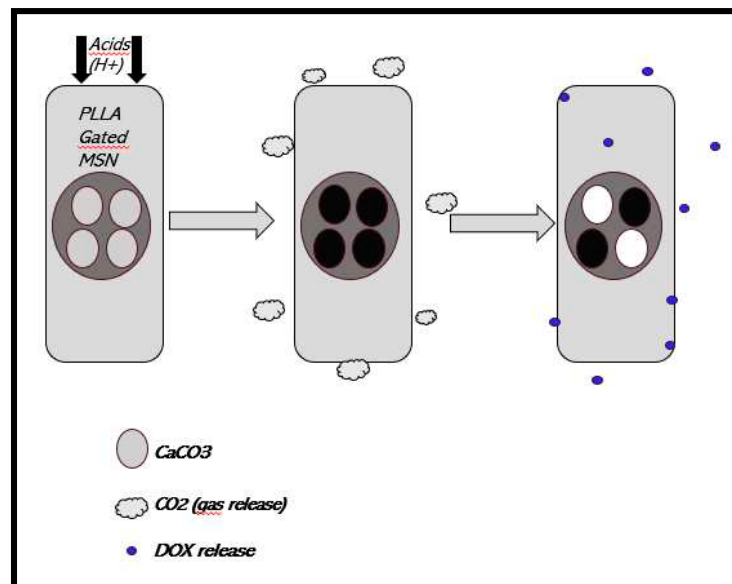


Figure 8. Scheme of DOX release mechanism. When protons infiltrates electrospun fibers, they react with the CaCO_3 which allows DOX release from MSN pores and the generation of CO_2 gas. This induces water entrance within fibers and DOX release. Inspired from reference [194].

Finally, there is the possibility to electrospun nanofibers for siRNA delivery to target specific cells [61]. Achille and al. [195] load-ed bioactive plasmid encoding for shRNA against Cdk2 into nanofibers. While fibers were degraded, plasmid DNA was released over 21 days. This prevented MCF-7 proliferation and decreased their viability.

3.4.7. Wound healing

To protect the wound, remove exudate and inhibit microorganisms, dressings are necessary. Pores, large air surface and stimulation of fibroblast cells are the advantages of dressings made from electrospun nanofibers. Treatment factors such as antibacterial factors, can be included in the matrix [45].

The introduction of natural extracts into the nanofibers makes it possible to enhance wound reduction without synthetic compounds. Natural compounds, such as plant-based extract or some phytoconstituents (Curcumin, Lemongrass) as said before, promote wound healing in distinct stages of the process. They prevent coagulation, inflammation, and induce re-epithelialization [156]. However, by only applying them on the wound, some inconvenience appears such as drying or softening the surrounding tissue. Consequently, introducing these components within electrospun scaffold is a promising alternative [156]. Aloe Vera has antioxidant properties, induces synthesis of collagen, hyaluronic acid and dermatan sulphate, essential components of the ECM, and promotes cell proliferation and migration [148]. Garcia-Orue et al. incorporated Aloe Vera and epidermal growth factors into PLGA nanofibers [196]. Fibroblast proliferation was stimulated, and the in vivo study showed a significantly increase in wound healing. Apitherapy is a potential target for wound healing as well. Synthesis of honey/PVA/chitosan and propolis nanofibers by electrospinning has antibacterial properties and promoted wound healing. Another study, on Waster rats, highlights the effects of *Garcinia mangostana* acetone extract, added into chitosan and poly (vinyl alcohol) polymer before electrospinning. It inhibits microbial colonization but also increase wound healing process [156].

Some polymers exhibit antibacterial properties. Among other materials, PAN nanofibers are one of them and their physiological mechanical properties make them a promising candidate for wound healing. Antibacterial activity against *S. aureus* have been seen as well in Cu/PAN nanofibers [45]. A patent, filed in 2019, defends the antifungal activity of microfibers made from PAN nanofibers. These are sprayed onto a TEFLON® plate and air-dried, then rinsed with acetone before being randomly stuck together in DMF [197]. Another example is presented in Ibrahim et al. work [198]. CMCS/PVA nanofibers containing gold nanoparticles (AuNP) were prepared by electrospinning and they showed the antibacterial activity of gold nanoparticles against Gram negative and positives bacteria were higher when encapsulated in the nanofibers than when they were alone and were non cytotoxic on A-549 cells. More recently, PCL-g-PAA electrospun nanofibers combined with GO-g-PTA nanosheets were studied for chronic wound care. The combination of the nanofibers absorbing body fluid and nanosheet's antibacterial properties enhances cell proliferation and wound healing process [199]. Quercetin is another natural compound presenting antioxidant, antiallergic, anticancer, antibacterial, and antifungal properties. It has been incorporated in PCL electrospun nanofibers and inhibited the formation of *C. albicans* biofilm [155].

Preventing scar formation is challenging, especially for deep burn victims. To prevent it, molecules, such as inhibitors of transforming growth factor- β 1, implicated into fibroblast proliferation deregulation, can be incorporated into the nanofibers to reduce scar formation [200]. By promoting early wound healing with ginsenoside-Rg3 added into PLGA fibers, the formation of hypertrophic scars at an advanced stage was prevented as well [201].

Diabetes might cause impaired repair mechanism and wound healing process [202]. To help wound healing, stimulation of collagen formation, re-epithelialization, vascularization, and inhibition of inflammatory reaction can be promoted by controlling the release of dimethylloxalylglycine that were loaded in aligned PLA nanofibers, improving wound healing of patients with diabetes [99].

Angiogenesis is required for both maintaining the survival of the new tissue as well as its growth. Chen et al. produced a PCL nanofibers scaffold with B and Co co-doped bioactive glass nanoparticles. The scaffold was not cytotoxic to bone marrow stromal cells ST-2 and their secretion of vascular endothelial growth factors was enhanced, and thus angiogenesis [203].

Finally, bifunctional matrices have shown great promise in healing tumor injury while preventing tumor recurrence. The addition of Cu₂S into PLA and PCL nanofibers resulted in increased skin tumor cell mortality, tumor growth inhibition after photothermal heating, while promoting proliferation and migration of healthy skin cells to heal the wound [204].

3.4.8. Implant coating

By coating implants with nanofibers, biocompatibility is improved and there is the possibility of adding new biological signals. To improve the expansion range of a stent, used to treat aneurysm, it was coated with PLCL nanofibers. Heparin and vascular endothelial growth factors were loaded in the nanofibers as well to prevent thrombus and induce endothelialization [205].

Nanofibers can be used as a barrier to prevent post-surgical adhesion with surrounding tissues during repair, induce osteogenesis, and prevent bacterial infection [206]. PCL nanofibers, after undergoing a cold plasma treatment, were functionalized with AMPS, and used to cover a polypropylene mesh for hernia repair. This was done to avoid adhesions post intra-abdominal surgery thanks to anticoagulant and antiadhesive properties. Those promising results on NIH3T3 cells needs further studies in vivo [207]. PAN nanofibers, loaded with doped Ag nanoparticles, have been used to capture *Escherichia coli*. The negative charges of the silver particles allowed immobilisation of antibodies helping to target specific pathogens by functionalizing the surface of the scaffold with anti-*E. coli*, only *E. coli* was captured, and *Staphylococcus aureus* did not bind it. Oppositely, on a scaffold functionalized with anti-*S. aureus*, only *S. Aureus* have been attached to the scaffold [206].

3.5. Combining microfluidic and electrospun fibers scaffold

Microfluidics allows the study of cells behavior by reproducing individual or combined physiological parameters. It ensures oxygen and nutrients distribution and wastes removal by performing laminar flow to better mimic blood vessels. Different cell types can interact, and gradient concentration can be applied through coating or in solution. Combining both microfluidic technology and 3D scaffold will greatly improve in vitro model in order to reduce cell culture artefact, increase possibilities of in vitro mimicking of biological process, then reducing the differences between pre-clinical and clinical studies [208]. Three different technologies are developed to combine microfluidics and 3D electrospun scaffold: lateral-flow models, electrospinning directly into a microfluidic channel and integration of electrospun fibers. The lateral-flow model is a microfluidic device like paper-based microfluidics. However, morphology and dimension can be monitored in contrast to paper. This technique is not suitable if shear stress is introduced. The second method is a direct electrospinning of the fibers inside the channel of the microfluidic device, but the channel must be at least 1mm large. With the third method, fibers are first electrospun, then the scaffold is cut, and cells seeded prior insertion in a 3D-printed microfluidic device. This process appears as the best suited as the scaffold with cells seeded on it can be removed and studied and the microfluidic device can be designed as desired.

Recently, Guida et al. introduced electrospun PCL fibres scaffold, in an open microfluidic chamber to study differentiation of mammospheres derived from human mammary glands into luminal cells [209]. Microfluidics allowed control of the micro-environmental factors and of the bioactive molecules exchanges while nanofibers were mimicking ECM, provided support for differentiation, proliferation, and migration. Combining electrospinning and microfluidics shows great promises for in vitro studies of getting closer from in vivo process.

4. Conclusion

In this review, we have described how 3D scaffolds could be designed by electrospinning, a nanofiber production method using electric forces. The theoretical aspect of the electrospinning process has been detailed. The multineedle electrospinning, which is an approach proposed for scaling up from laboratory to production scale, has been presented. Electrospun scaffolds are used in many fields such as protection, production of electrode materials and sensors, filtration, data storage and transport in informatics, textile production, adsorption, decomposition of pollutants, and biomedical applications. We reviewed the possible applications involving electrospun fibers in different domains of medicine and biotechnologies. Among the materials used for electrospinning, PAN is the main carbon fibers precursors since it has a high melting point and a great carbon yield. Fibers possess good resistance, low density, high stiffness, lightweight, have amazing biocompatibility properties and allow possible use of low toxic solvents. However, PAN electrospun nanofibers are not yet widely widespread for production of scaffolds employed for cell culture and biological studies. We provided bibliographic data on the way PAN could be electrospun into fibers and presented the subsequent treatments prior to applications. The applications of exPAN fibers were described in the biomedical field, and how 3D scaffolds are particularly attractive for cell growth. The possibility of tuning mechanical properties of the scaffold independently from other parameters offers new opportunities in research, aiming to understand mechanosensing process in a 3D environment and effect on cellular behavior. As well, 3D nanofibers scaffold represents a promising material for future developments helping with drug screening, cell therapy, and bioproduction, in a biomimicking and highly controllable in vitro microenvironment.

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